

## The Relation of Salivary Constituents (Urea, Calcium and Phosphorous) to Root Caries among Overweight and Obese Adults Aged 55-65 Year-Old at Textile Factory in Mosul City

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### Key words

overweight, obesity, root caries, salivary urea, calcium and phosphorous.

### Abstract

The purpose of this study was to disclose the relationship of salivary urea, calcium and phosphorous levels with root caries occurrence among overweight and obese adults aged 55-65 year-old at Textile Factory in Mosul City. All subjects aged 55-65 year-old (thirty five subjects) at Textile factory in Mosul city who fitted the study criteria took part in the current study. Weight status was determined by using the Body Mass Index (BMI). Root caries was recorded according to the criteria of WHO (1997). Unstimulated whole saliva was collected then salivary samples were subjected for biochemical analysis. Salivary urea, calcium, and phosphorous were determined colorimetrically by using the spectrophotometer. Results revealed that salivary urea and phosphorous levels were higher among obese and overweight subjects than non-obese with highly significant difference. Also calcium level was significantly elevated among obese compared with non-obese. Sound root surface value was higher among obese than non-obese and overweight subjects with significant difference. On the other hand overweight and obese subjects revealed lower decayed root surface value than non-obese though statistical difference was not significant. As a conclusion obese subjects with good general health might experience reduced root caries severity that might partly be due to changes in salivary constituents. Therefore future studies should address which factors specific to obese might be protective against root caries. Also there is a need for further studies with larger sample size and another sample distribution that include underweight, normal weight, overweight and obese to get more precise and clear results.

### Introduction

Overweight and obesity have become a global problem <sup>(1)</sup>. Body composition changes with advancing age with an increase in fat mass and loss in muscle mass which is termed sarcopenic

obesity <sup>(2)</sup>. Overweight also defined as having more body fat than is optimally healthy but it is considered as a state of pre obesity <sup>(3)</sup>. Obese people are at higher risk for heart diseases, diabetes mellitus, cancer, joint diseases, and psychological problems, etc <sup>(4)</sup>. In old people it is difficult to establish an ideal BMI that

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minimizes risk of mortality or morbidity. The Nutrition Screening Initiative recommended a Body Mass Index between (22-27 kg/m<sup>2</sup>) in the elderly <sup>(5)</sup>. Regarding oral diseases, overweight and obesity were found to influence many aspects of oral health <sup>(6)</sup>. Root surface caries can be defined as soft progressive lesion that is found anywhere on the root surface that has lost epithelial tissue attachment and is exposed to the oral environment; however, root caries is not necessarily a sequel of gum recession in all persons <sup>(7,8)</sup>. The etiologic mechanism of root caries doesn't, in principle, differ from coronal caries <sup>(9)</sup>. Occasionally, the composition of bacterial plaque flora on root surfaces differs from that on enamel surfaces in that *Actinomyces* and *Lactobacilli* are the main root caries pathogens in addition to acidogenic microorganisms <sup>(10)</sup>. Root lesions usually progress more quickly than do enamel lesions, this is due to the low degree of mineralization of root cementum, and also because of the difficulties in keeping root surfaces clean with routine brushing of teeth <sup>(11)</sup>. Several studies among children and adolescents found that overweight and obese subjects are more likely to have dental caries than the non-obese ones <sup>(12-14)</sup>. Another study among adults found that obesity could increase the risk for dental caries probably because obese subjects tend to neglect their oral health <sup>(15)</sup> also they used to eat frequent sugary snacks <sup>(16)</sup>. Pannunzio et al <sup>(17)</sup> added that changes in salivary constituents among obese provided a favorable condition for dental caries. They carried out a study among school children and found a significant elevation in salivary protein with a significant reduction of phosphate level in saliva of obese and overweight children compared with control. However, calcium level revealed no significant difference among the three groups. No studies could be found regarding root caries among overweight and obese adults. Also there is no previous Iraqi studies concerning the relation of certain salivary elements (urea, calcium and phosphorous) with root caries among overweight and obese individuals, therefore, this study was conducted.

## Subjects and Methods

The sample consisted of all subjects of both genders aged 55-65 year-old at Textile factory in Mosul city. They should be non-smoker, with no medical history that compromises salivary secretory mechanism (depending on the medical report supplied by the medical unit at the factory), shouldn't take any medications with xerogenic effect or any nutritional supplementation, and shouldn't wear any fixed or removable dental prostheses. The collection of unstimulated salivary samples was performed according to the instructions cited by Tenovuo and Lagerlöf <sup>(18)</sup>. Then salivary samples were subjected for biochemical analysis at the College of Veterinary and College of Dentistry, University of Mosul. Salivary urea, calcium, and phosphorous were determined colorimetrically by using ready kits supplied by (BioMérieux sa, France) using the spectrophotometer (Cecil Instrument Limited CE 1021, England). Root caries was recorded according to the criteria of WHO (1997) <sup>(19)</sup>. Weight status was determined by using the Body Mass Index (BMI) which can be obtained by dividing weight in Kilogram by height in meter squared <sup>(1)</sup>. BMI was divided into three categories which include non-obese (<25 Kg/m<sup>2</sup>), overweight (≥25-<30 Kg/m<sup>2</sup>), and obese (≥30 Kg/m<sup>2</sup>) <sup>(20)</sup>. Data analysis was conducted through the application of the Statistical Package for Social Science (SPSS version 12). Analysis of variance (ANOVA) test, Least Significant Difference (LSD) test and Multiple Linear Regression (MLR) test were applied. The confidence limit was accepted at 95% (P <0.05).

## Results

Sample distribution is shown in Table 1. Non-obese and obese subjects revealed equal percentage (37.14%) while the percentage of overweight subjects was (25.71%). Table 2 reveals highly significant difference in mean salivary urea level among BMI categories (F= 8.42; P<0.01). Whereas calcium showed

statistically non significant difference among BMI categories ( $P>0.05$ ). Concerning phosphorous level, it revealed highly significant difference among the three categories ( $F= 7.84$ ;  $P<0.01$ ) Further investigation using Least Significant Difference (L.S.D.) test as shown in Table 3 revealed that urea level was higher among obese than non-obese with highly significant difference (m.d.=-21.13,  $P<0.01$ ) also it was higher among obese than overweight with significant difference (m.d.=-12.90,  $P<0.05$ ). Furthermore Least Significant Difference (L.S.D.) test showed that calcium level was significantly higher among obese than non-obese (m.d.=-1.78,  $P<0.05$ ) and overweight individuals (m.d.=-1.82,  $P<0.05$ ). Also phosphorous level was higher among obese than non-obese with highly significant difference (m.d.=-5.75,  $P<0.01$ ) also phosphorous was significantly higher among obese than overweight (m.d.=-4.02,  $P<0.05$ ). Root caries according to BMI categories is seen in Table 4. Sound root surface value (SS) showed significant difference among the three categories ( $F=3.80$ ;  $P<0.05$ ). On the other hand decayed root surface mean value (DS) was lower among overweight and obese than non-obese though statistical difference was not significant. In addition Least Significant Difference (L.S.D.) test showed that sound root surface value was significantly higher among obese than non-obese (m.d.=-18.77,  $P<0.05$ ) and overweight individuals (m.d.=-24.16,  $P<0.05$ ) (Table 5). Results of Multiple Linear Regression (MLR) test for sound root surface (SS) (dependent variable) explained by salivary constituents (independent variables) is seen in Table 6. Among non-obese subjects a complete correlation coefficient R between them was 0.61 with coefficient of determination  $R^2$  value of 0.37 indicating that 37% of changes occurred in (SS) are explained by salivary constituents (urea, calcium, phosphorous). For all entered constituents beta coefficient slopes were not statistically significant ( $P>0.05$ ). Among overweight a complete correlation coefficient R between them was 0.72 with coefficient of determination  $R^2$  value of 0.51 indicating that 51% of changes

occurred in (SS) are explained by salivary constituents (urea, calcium, phosphorous). Also for all entered constituents beta coefficient slopes were not statistically significant ( $P>0.05$ ). Whereas among obese subjects complete correlation coefficient R was 0.85 with coefficient of determination  $R^2$  of 0.72 indicating that 72% of changes occurred in (SS) are explained by salivary constituents (urea, calcium, phosphorous). The only highly significant beta coefficient was found for calcium with positive trend of effect. Results of Multiple Linear Regression (MLR) test for decayed root surface (DS) (dependent variable) explained by salivary constituents (independent variable) is shown in Table 7. Among non-obese subjects a complete correlation coefficient R between them was 0.41 with coefficient of determination  $R^2$  value of 0.16 indicating that 16% of changes occurred in (DS) are explained by salivary constituents (urea, calcium, phosphorous). For all entered constituents beta coefficient slopes were not statistically significant ( $P>0.05$ ). No statistics was computed for overweight because they had no root caries (see Table 4). For obese subjects complete correlation coefficient R was 0.83 with coefficient of determination  $R^2$  value of 69%. The only significant beta coefficient was recorded for calcium with positive trend of effect.

## Discussion

Saliva is essential for preserving oral health <sup>(21)</sup>. Regarding the protection against dental caries, salivary flow rate, buffer capacity (e.g. urea), calcium, phosphorous and fluoride concentration are essential <sup>(22)</sup>. Obesity is currently a rapidly growing form of malnutrition <sup>(23)</sup>. Inadequate nutritional status may influence saliva composition, which in turn affects the process of tooth demineralization <sup>(17)</sup>. Findings of the current study revealed higher salivary urea among obese and overweight than non-obese with highly significant difference. No study could be found regarding salivary urea level among obese and overweight to compare the results with.

However, since urea is a byproduct of protein metabolism<sup>(24)</sup> the study result is partially agreed with Pannunzio et al<sup>(17)</sup> study among school children .It was found that obese subjects are more likely to have either lower blood concentrations or lower bioavailability of minerals and/or vitamins so they need multivitamin and mineral supplementation<sup>(25)</sup>. However, in the current study results revealed that calcium was significantly higher among obese than overweight and non-obese groups. This finding disagreed with Pannunzio et al<sup>(17)</sup> results. Higher calcium level among obese in the current study probably related to the fact that obese subjects experience metabolic and hormonal disturbances like an increase in parathyroid hormone release which in turn leads to increased release of calcium from bone and reduction of calcium loss from kidneys so that blood calcium level rises<sup>(26)</sup> so as salivary calcium level since saliva is considered as a mirror of serum<sup>(27)</sup>. Salivary phosphorous level in this study was higher among obese and overweight than non-obese with highly significant difference. This finding is contradicted with Pannunzio et al<sup>(17)</sup> study. This variation with other studies probably related to differences in sample size, criteria of sample selection and classification, collection method of saliva, age group, geographic location and diet<sup>(28)</sup>. Also in this study no dietary analysis was performed especially regarding calcium, phosphorous, protein and lipid intake. Concerning root caries experience obese subjects revealed significantly higher sound root surface mean value than non-obese on the other hand they showed lower decayed root surface value than non-obese but with no significant difference. This finding disagreed with other studies<sup>(12-15)</sup>. Contradiction with these studies probably because our study sample was healthy with no systemic diseases and not used any medications that affect saliva secretion and consequently oral health<sup>(29)</sup> so they had less decayed root surfaces value but higher sound root surfaces value. Another explanation is higher salivary urea, calcium and phosphorous among obese that were reported to have a protective effect against

dental caries by several studies<sup>(30-33)</sup> thereby reducing the susceptibility of the exposed root surfaces and increasing their resistance to dental caries. This is probably because salivary urea as a buffer system causes pH rise thereby enhancing tooth surface remineralisation<sup>(34)</sup>. Also it has the ability to modify the cariogenic potential of the oral environment by fostering an environment less conducive to the emergence of acidogenic and aciduric species but more inductive to mineral deposition<sup>(32)</sup>. Also salivary calcium plays an essential role in increasing resistance of the outer enamel surface to acid dissolution and enhancing remineralization of the initial carious lesion<sup>(18)</sup>. In addition phosphorous might enhance remineralization and increases tooth resistance to acid attack also it acts as buffer system<sup>(35)</sup>. This is further supported by highly significant Beta coefficient for calcium on sound root surface with positive trend of effect that is found in the current study. It could be concluded that obese subjects with good general health might experience reduced root caries severity that might partly be due to changes in salivary constituents which favor less root caries severity. Therefore, future studies should address which factors specific to obese might be protective against root caries. It is worth to mention that non-obese category includes normal weight and underweight. Therefore, it is recommended to carry out further study with larger sample size and another sample distribution that include underweight, normal weight, overweight and obese to get more precise and clear results.

Table(1):- Sample distribution according to body weight categories.

BMI categories	No.	%
Non-obese ( $<25 \text{ Kg/m}^2$ )	13	37.14
Over weight ( $\geq 25 - < 30 \text{ Kg/m}^2$ )	9	25.71
Obese ( $\geq 30 \text{ Kg/m}^2$ )	13	37.14
Total	35	100

Table (2):- Salivary constituents (Mean  $\pm$ S.D.) according to body weight categories.

Variable	Non-obese (<25 Kg/m <sup>2</sup> )			Over weight ( $\geq$ 25 -<30 Kg/m <sup>2</sup> )			Obese ( $\geq$ 30 Kg/m <sup>2</sup> )			ANOVA test d.f.=2	
	No.	Mean	$\pm$ SD	No.	Mean	$\pm$ SD	No.	Mean	$\pm$ SD	F-value	P-value
Urea	13	44.46	15.81	9	52.69	12.86	13	65.59	10.26	8.42	0.001**
Calcium	13	8.67	1.92	9	8.64	1.21	13	10.45	2.44	3.30	0.050
Phosphorous	13	9.48	3.88	9	11.22	2.89	13	15.24	4.18	7.84	0.002**

\*\* Highly Significant

Table(3):- Least Significant Difference (L.S.D) of salivary constituents according to body weight categories.

Variable	BMI categories	Mean difference (m.d.)	Standard Error	Significancy
Urea	Non-obese-Obese	-21.13	5.18	0.00**
	Over weight-Obese	-12.90	5.73	0.03*
	Non-obese-Over weight	-8.23	5.73	0.16
Calcium	Non-obese-Obese	-1.78	0.78	0.03*
	Over weight-Obese	-1.82	0.867	0.04*
	Non-obese-Over weight	0.03	0.867	0.97
Phosphorous	Non-obese-Obese	-5.75	1.48	0.00**
	Over weight-Obese	-4.02	1.64	0.02*
	Non-obese-Over weight	-1.73	1.64	0.30

\*Significant

\*\* Highly Significant

Table (4):- Root caries (Mean  $\pm$ S.D.) according to body weight categories.

Root Caries	Non-obese (<25 Kg/m <sup>2</sup> )			Over weight ( $\geq$ 25-<30 Kg/m <sup>2</sup> )			Obese ( $\geq$ 30 Kg/m <sup>2</sup> )			ANOVA test df=2	
	No.	Mean	$\pm$ SD	No.	Mean	$\pm$ SD	No.	Mean	$\pm$ SD	F-value	P-value
SS	13	25.61	12.60	9	20.22	27.00	13	44.38	25.91	3.80	0.03*
DS	13	0.46	0.52	9	0.00	0.00	13	0.31	0.75	1.83	0.18

\*Significant

Table (5):- Least Significant Difference (L.S.D) of root caries according to body weight categories.

Root Caries	BMI categories	Mean difference (m.d.)	Standard Error	Significancy
SS	Non-obese- Obese	-18.77	8.71	0.04*
	Over weight-Obese	-24.16	9.63	0.02*
	Non-obese-Over weight	5.39	9.63	0.58
DS	Non-obese-Obese	0.15	0.22	0.49
	Over weight-Obese	-0.31	0.24	0.21
	Non-obese-Over weight	0.46	0.24	0.07

\*Significant

Table (6):- Multiple Linear Regression of sound root surface (SS) explained by certain salivary constituents according to body weight categories.

Salivary constituents	Non-obese (<25 Kg/m <sup>2</sup> )				Over weight (≥25 -<30 Kg/m <sup>2</sup> )				Obese (≥30 Kg/m <sup>2</sup> )			
	β (slope)	S.E.	t	P-value	β (slope)	S.E.	t	P-value	β (slope)	S.E.	t	P-value
Urea	0.26	0.48	0.54	0.60	-3.71	2.37	1.57	0.18	-1.16	0.93	1.24	0.25
Calcium	0.22	2.66	0.08	0.94	-23.38	22.53	1.04	0.35	7.63	1.92	3.99	0.003**
Phosphorous	0.91	2.36	0.39	0.71	16.69	10.28	1.62	0.17	3.81	2.31	1.65	0.13
R=0.61 R <sup>2</sup> =0.37				R=0.72 R <sup>2</sup> =0.51				R=0.85 R <sup>2</sup> =0.72				

\*\* Highly Significant

Table (7):- Multiple Linear Regression of decayed root surface (DS) explained by certain salivary constituents according to body weight categories.

Salivary constituents	Non-obese (<25 Kg/m <sup>2</sup> )				Obese (≥30 Kg/m <sup>2</sup> )			
	β (slope)	S.E.	t	P-value	β (slope)	S.E.	t	P-value
Urea	0.0007	0.02	0.03	0.98	-0.06	0.03	-2.16	0.06
Calcium	-0.14	0.13	-1.12	0.29	0.16	0.06	2.70	0.02*
Phosphorous	0.02	0.11	0.21	0.84	0.04	0.07	0.58	0.58
R=0.41 R <sup>2</sup> =0.16				R=0.83 R <sup>2</sup> =0.69				

\* Significant

## References

- 1-Insel P, Turner RE, Ross D. Discovery nutrition. Jones and Bartlett publishers. USA; 2003. p. 269-293.
- 2-Sezginsoy B, Ross K, Wright JE, Bernard MA. Obesity in the elderly, survival of the fit or fat. J Okla state Med Assoc 2004; 97(10): 437-9.
- 3-Flegal Katherine M, Carroll Margaret D, Johnson Clifford L, Johnson CL. Prevalence and Trends in Obesity Among US Adults, 1999-2000. JAMA 2002; 288 (14): 1723-1727.
- 4-Roth RA and Townsend CE. Nutrition and Diet therapy. 8<sup>th</sup> ed. Thomson Delmar Learning. USA; 2003. p. 295-309.
- 5-Schlenker ED. Nutrition in aging. 3<sup>rd</sup> ed. WCB McGraw-Hill Co.; 1998. p. 295-325.
- 6-Mathus-Vliegen EM, Nikkel D, Brand HS. Oral aspects of obesity. Int Dent J 2007; 57(4): 249-56 [IVSL].
- 7-Chalmers JM. Geriatric oral health issues in Australia. Int Dent J 2001; 51 (3 suppl): 188-199 [IVSL].
- 8-Gupta B, Marya CM, Juneja V, Dahiya V. Root caries, An aging problem. Internet J Dent Sci 2007; 5 (1): 1-10.
- 9-Feng L, Gao X. The dynamics of pH, free calcium and total proteins in root plaque fluid and their relationships to the cariogenic potential. Zhonghua Kou Qiang Yi Yue Za Zhi 2000; 35(2): 132-4.
- 10-Zhang T, Zhang Y, Zhang C, Yang S. Pathogen of root surface caries in the elderly. Chin Med J 2001; 114 (7): 767-768.
- 11-Konig KG and Navia JM. Nutritional role of sugars in oral health. Am J Clin Nutr 1995; 62(suppl): 275-83.
- 12-Bailleul-Forestier I, Lopes K, Souames M, Azoguy-Levy S, Frelut ML, Boy-lefevre ML. Caries experience in a severely obese adolescent population. Int J Paediatr Dent 2007; 17(5): 358-63.
- 13-Sadeghi M and Alizadeh F. Association between Dental Caries and Body Mass Index-For-Age among 6-11-Year-Old Children in Isfahan in 2007. J Dent Res 2007; 1(3).
- 14-Alm A, Isaksson H, Fähræus C, Koch G, Andersson-Gäre B, Nilsson M, Birkhed D, Wendts LK. BMI status in Swedish children and young adults in relation to caries prevalence. Swed Dent J 2011; 35(1):1-8.
- 15-Griffin SO, Barker LK, Griffin PM, Cleveland JL, Kohn W. Oral health needs among adults in the United States with chronic diseases. JADA 2009; 140 (10): 1266-1274.
- 16-Basavaraj P, Khuller N, Khuller RI, Sharma N. Caries Risk Assessment and Control. J Oral Health Comm Dent 2011, 5(2): 58-63.
- 17-Pannunzio E, Amancio OMS, Vitale MSS, de Souza DN, Mendes FM, Nicolau J. Analysis of the stimulated whole saliva In overweight and obese school children. Rev Assoc Med Bras 2010; 56(1): 32-6.
- 18-Tenovuo J and Lagerlöf F. Saliva. In Textbook of clinical cardiology. Etd. By Thylstrup A and Fejerskov O. 2<sup>nd</sup> ed Munksgaard Copenhagen; 1994. p. 17-43.
- 19-WHO. Oral health surveys basic methods. 4<sup>th</sup>ed. World Health Organization. Geneva, Switzerland, 1997.
- 20-Wu FCW, Tajar A, Pye SR, Silman AJ, Finn JD, O'Neil TW, Bartfai G, Casaneuva F, Forti G, Giwercman A, Huhtaniemi IT, Kula K, Punab M, Boonen S, Vandershueren D, EMAS group. Hypothalamic pituitary testicular axis distribution in older men is differentially linked to age and modifiable risk factors. J Clin Endocrin Metab 2008; 93 (7): 273-45.
- 21-Lenander-Lumikari M, Loimaranta V. Saliva and dental caries. Adv Dent Res 2000; 14: 40-47.
- 22-Lagerlöf F, Oliveby A. Caries-protective factors in saliva. Adv Dent Res 1994;8(2):229-38.
- 23-Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity worldwide. Inter Br Med J 2000; 320(6):1-6.
- 24-Bishop ML, Duben-Engelkirk JL, Fody EP. Clinical chemistry: principle, procedures, correlation. 4<sup>th</sup> ed. Lippincott Williams and Wilkins, Philadelphia, 2000 Bishop ML, Duben-Engelkirk JL, Fody EP. Clinical chemistry: principle, procedures, correlation. 4<sup>th</sup> ed. Lippincott Williams and Wilkins, Philadelphia, 2000.
- 25-Li Y, Wang C, Zhu K, Feng RN, Sun CH. Effects of multivitamin and mineral supplementation on adiposity, energy expenditure and lipid profiles in obese Chinese women. Int J Obes 2010; 34(6): 1070.
- 26-Holecki M, Chudek J, A.Wie,cek, Titz-Bober M, Duława J. The serum level of fibroblast growth factor-23 and calcium phosphate homeostasis in obese perimenopausal women. Int J Endocrinol 2011; 2011: 707126.

- 27-Kaufman E, Lamster IB. The diagnostic application of saliva. *Int Am Assoc Dent Res* 2002; 13 (2): 197-212.
- 28-Siqueira Jr WL, Nicolau J. Stimulated whole saliva components in children with Down Syndrome. *Spec Care Dentist* 2002; 22(6): 226-30.
- 29-Mauriello SM. Risk modeling for root caries and mortality in older adults. A Ph.D. thesis, Adult and community college education, North Carolina state university, 2004.
- 30-Ryberg M, Moller C, Ericson T. Saliva composition and caries development in asthmatic patients treated with beta-adrenoceptor agonists: a 4-year follow up study. *Scand J Dent Res* 1991; 99: 212-8.
- 31-Woltgens JH, Gruythuysen RJ, Linden LW, Geraets WG. Cariogenic changes in dental enamel of boys and girls in relation to salivary properties. II. Radiological examination. *J de Biol Buccale* 1992; 20: 235-40.
- 32-Sulaiman AWR. Quantitative measurement of urea content in saliva, acquired pellicle and dental plaque in relation to dental caries susceptibility in human adults. A Ph.D. Thesis. College of Dentistry, University of Baghdad, 2000.
- 33- El-Samarrai SK. Major and trace elements of permanent teeth and saliva among a group of adolescent in relation o dental caries, gingivitis and *Mutans Streptococci*. A Ph.D. Thesis. College of Dentistry, University of Baghdad, 2001.
- 34-Shu M, Morou-Bermudez E, Suárez-Perez E, Rivera-Miranda C, Browngardt CM, Chen YYM, Magnusson I, Burne RA. The relationship between dental caries status and dental plaque urease activity. *Oral microbial Immunol* 2007; 22 (1): 61-6.
- 35-Ten Cate BT. The role of saliva in mineral equilibrium, caries and calculus formation. In saliva and oral health ed. By Edger W. and O'Mullane D. 2<sup>nd</sup> ed. Thanet Ltd. London; 1996 . p. 123-136.