6-1976

Effects of Cariogenic and Noncariogenic Diets on the Concentration of the Parotid Hormone Releasing Factor in Rat Hypothalami

Melva Joan Brown

Abstract

EFFECTS OF CARIOGENIC AND NONCARIOGENIC DIETS
ON THE CONCENTRATION OF THE PAROTID HORMONE
RELEASING FACTOR IN RAT HYPOTHALAMI

by

Melva Joan Brown

Hypothalamic and cortical extracts from various cariogenically grouped rats which had been maintained on either a Purina diet or a high sucrose diet (HSD) for three months were infused intravenously into 28 days old rats to determine if there was a titer of parotid hormone releasing factor (PRF) capable of facilitating fluid movement (FM) in the teeth. The hypothalamic and cortical extracts obtained from Purina-fed rats with 0-caries and the hypothalamic extracts from HSD-fed rats conclusively demonstrated FM stimulatory ability. Other Purina-fed groups were essentially biologically ineffective, however, extracts from HSD-fed caries groups including scores 11 - 40 demonstrated extremely potent FM stimulating ability. The extract from the 1 - 10 caries group for the HSD-fed rats was an effective stimulator but only one-third as potent as the physiologically homeostatic 0-caries group. Extracts from groups containing more than 40 caries did not demonstrate stimulatory ability at the tested doses, suggesting that synthesis of PRF is not adversely affected within an optimal range when caries develop.

LOMA LINDA UNIVERSITY

Graduate School

EFFECTS OF CARIOGENIC AND NONCARIOGENIC DIETS ON THE CONCENTRATION OF THE PAROTID HORMONE RELEASING FACTOR IN RAT HYPOTHALAMI

by

Melva Joan Brown

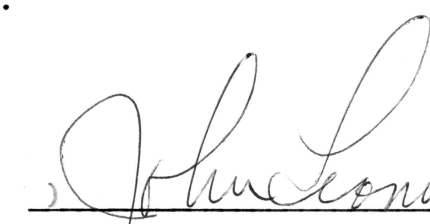
A Thesis in Partial Fulfillment

of the Requirements for the Degree Master of Science

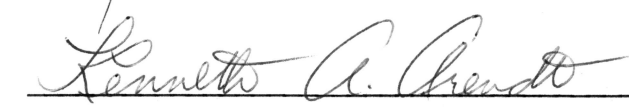
in the Field of Physiology

June 1976

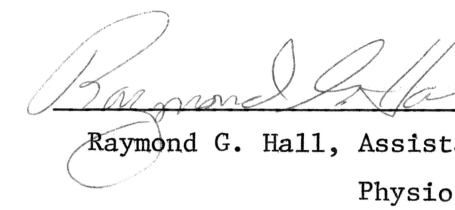
Each person whose signature appears below certifies that this thesis in his opinion is adequate, in scope and quality, as a thesis for the degree Master of Science.



John Leonora, Professor of Physiology



Kenneth A. Arendt, Professor of Physiology



Raymond G. Hall, Assistant Professor of Physiology

ACKNOWLEDGEMENT

My sincere appreciation goes to Dr. John Leonora for his superb guidance and assistance; to Dr. Ralph Steinman for the reading of fluid movement in the teeth making it possible to determine and analyze my data; to Dr. Kenneth Arendt and Dr. Raymond Hill, members of my Guidance Committee; and to Ronald Fioretti for his technical assistance.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter 1

INTRODUCTION

W. D. Miller in the 1890's incubated a tooth in a mixture of saliva and bread and observed that salivary bacteria fermented the carbohydrate producing sufficient bacterial acid to decalcify the tooth. As a result, Miller proposed that bacteria were the etiologic agents of dental caries. Dental researchers today have virtually left unchallenged his bacterial etiology of caries theory (Gibbons, 1968). In 1964 Gibbons reviewed the evidences for bacteria as the causative factor in caries, and reaffirmed Miller's views. Socransky (1972) suggested that in addition to essential microbiota, it is necessary to have a tooth which is susceptible to decay and a diet conducive to cariogenesis. Keyes (1968) advises that the etiological factors associated with caries be discussed on three planes of reference:

1. Reaction at the molecular level: i.e. the chemical interaction between the inorganic and organic compounds of the teeth and bacterial end products.

2. Precursory factors: microorganisms, dietary substrates, certain physiochemical properties of the tooth.

3. Modifying influences: shape of teeth (e.g. retention sites), eating patterns, quantity and quality of salivary secretions, oral clearance, alimentary canal microbiota, etc.

According to Scherp (1971) dental caries result from localized progressive decay of the teeth initiated by demineralization of the

outer surface of the tooth due to organic acids produced locally by bacteria that ferment deposits of dietary carbohydrates. This 1971 explanation for caries varies little from that of the 1890's given by Miller. There are still no clearly evident therapeutic leads that promise to do more, at best, than arrest a carious lesion once it is clinically detectable.

Even medical dictionaries concur with the acidogenic theory proposed by Miller. Dental caries is the lysis of hydroxyapatite-like crystals in enamel, cementum, and dentin by acids of bacterial origin with subsequent loss of the organic matrices (Blakiston, 1956; Dorland, 1965; Stedman, 1961). Many researchers believe that caries begin in the enamel and only secondarily involve the other tooth structures (Zipkin, 1968). Of the four basic types of caries delineated by Socransky (1972) only one is believed to penetrate the root surfaces first.

Dental literature indicates that caries research is overwhelmingly concerned with enamel protection; saliva, plaque, diet and plaque, acid formation in mouth, bacteria flouridation as well as topical application of fluorides, etc. Caries research has become a search for new means to increase the caries resistance of teeth, to reduce the cariogenicity of foodstuffs and to check the deleterious activities of cariogenic bacteria (Scherp, 1971).

There is evidence, however, that internal changes occur before the enamel is affected. Sharpenak (1972), Gwinnett (1971), Brannstrom, M. and Linda, P. O. (1965), Steinman, R. R. and

Leonora, J. (1971) give evidence that caries indicate some physiological failure.

In 1968 Leonora and Steinman demonstrated an hypothalamic-parotid-endocrine axis (HPEA) using a fluorescent dye technique to study the movement of fluid through the odontoblastic tubules in the dentin of rat molars. The tooth was then shown to be affected by an endocrine control system that was dependent on the hypothalamus and the parotid gland. The presence of a fluid transport system associating the pulp with the enamel had been demonstrated by earlier investigators, i.e. Bodecker (1923, 1929), Bodecker and Applebaum (1931), Bodecker and Solewen (1937, 1946), Fish (1926, 1927a, 1927b), von Beust (1912, 1931) and Lefkowitz (1943) using different dyes. Histological evidence was presented as early as 1854 by Kölliker as well as Bodecker (1911) and von Beust (1912). More recently Volker and Sognnaes (1940), Armstrong and Barnum (1948), and Sognnaes, Shaw and Bogoroch (1955) have used radio active isotopes to demonstrate this fluid transport system. Using thermal induction Kreudenstein (1955), Kreudenstein and Steuben (1955, 1956) and Steuben and Kreudenstein (1956) have demonstrated fluid movement (FM) in freshly extracted teeth. Bergman and Linden (1965), Atkinson (1947, 1948, 1969) and Pincus (1958) also elaborate on the odontoblast controlled enamel-dentin complex.

The concept of a higher control superceding the buccal cavity's autonomous activity seemed novel. Here appeared to be a new approach to preventive and/or corrective tooth decay therapy.

Researchers have known that cavities develop readily in individuals who:

1. Subsisted on a high level of sucrose in their diet;

2. were endowed with a bountiful supply of microbes, in particular a certain species of Streptoccus;

3. and had the optimal dental milieu (Scherp, 1971).

This host-parasite-environment complex was imperative. Since caries development was shown to be co-dependent on all three factors, elimination or control of either of these variables could prove to be beneficial for cavity control.

When one considers the susceptible tooth itself, previous studies by Steinman, R. R. and Hardinge, M. G. (1958), Steinman, R. R. (1961), Sanchez, A., Steinman, R. R. and Leonora, J. (1970), and Steinman, R. R. and Leonora, J., (1971) show that active FM in the odontoblastic processes of rat's dentin had been suppressed in animals on a cariogenic diet. When adequate FM is maintained, the tooth apparently loses its susceptibility to dental caries. A number of additives and compounds were used by these researchers to demonstrate and assist FM in the tooth. Leonora, J. and Steinman, R. R. (1968) reported that the chain of hierarchy in the command process involved the hypothalamus releasing a parotid hormone releasing factor (PRF) which stimulates FM in the odontoblastic process hence preventing tooth decay. It was demonstrated by these researchers that the hypothalamus was probably involved with control of FM in the tooth.

The purpose of this investigation was to examine the probable alterations of the concentration of the hypothalamic PRF in rats which had been fed a cariogenic diet for three months. A comparison of concentrations of the hormone in the hypothalamus was made by classifying the glands according to an arbitrary caries score.

Chapter 2

METHODS AND MATERIALS

Extraction of Rat Hypothalamic and Cortical Tissue

Hypothalami and comparable weights of cortical tissue were excised from 248 male Sprague Dawley rats obtained from the Simonsen Laboratory Inc. in Gilroy, California. Forty-three were maintained on a Purina diet and 205 on a high sucrose diet (HSD) for three months. The number of cavities present in each rat was observed and the hypothalami and cortices were grouped according to the cavities observed. The categories were: for HSD 0, 1-10, 11-20, 21-30, 31-40, 41-50, 51 and above; and for Purina-fed 0, 1-5, 6 and above. The hypothalami were homogenized with 10 ml. of cold 0.01 N HCl/100 g. of fresh tissue. The homogenized tissue was then neutralized to a pH of 7 with 0.1 N NaOH. The pH adjustments were accomplished with a Corning pH meter model #12. The mixture was then centrifuged at 3,000 RPM for 30 minutes. The supernatant was removed and the precipitate washed with distilled water and centrifuged four times for each category. The collective supernatant for each category was freeze dried. A comparable procedure was followed for the cortices. The equivalent weight of dry tissue to fresh tissue is shown in Table 1.

Bioassay Procedure

Male, 21 days old Sprague Dawley rats strain B from the Simonsen Laboratory Inc., Gilroy, California, were maintained on a

Table 1. Equivalent Weights of Dried to Fresh Hypothalamic and Cortical Tissues

Tissue Type	Fresh Tissue Weight (g)	Dried Tissue Weight (g)	$\frac{\text{Dried Tissue}}{\text{Fresh Tissue}}$ (g/g)
Hypothalamus			
Purina Caries			
0	6.461	0.453	0.070
1-5	3.861	0.287	0.074
6 & Above	2.457	0.213	0.087
HSD Caries			
0	19.371	1.178	0.061
1-10	13.965	0.994	0.071
11-20	4.904	0.343	0.070
21-30	4.679	0.342	0.073
31-40	3.330	0.219	0.066
41-50	2.985	0.281	0.094
51 & Above	1.356	0.113	0.083
Cortex			
Purina Caries			
0	6.119	0.603	0.099
HSD Caries			
0	16.620	1.156	0.070
51 & Above	1.256	0.174	0.139

Purina diet for an additional week in the Animal Care Facilities of the Loma Linda University Medical Center and then used to bioassay the hypothalamic and cortical extracts of the categories of preparations listed above.

The rats were anesthetized with approximately 0.075 ml/100 g body weight, sodium pentobarbital, weighed, numbered and placed on the dorsal surface with head toward researcher, forelimbs extended and maintained in position with masking tape. One ml. of the fluorescent dye, acriflavine hydrochloride, was then injected intraperitoneally. Ten minutes later, the right subclavian vein was exposed and intravenous injections of either 0.5 ml. of hypothalamic or 0.5 ml. of cortical extract reconstituted in a moral saline solution were infused. The dosage ranged from 0.1 ug. equivalent (eq.) fresh tissue decreased by 1/100 to 0.01 ug. eq. and from 0.01 ug. eq. decreased by 1/2 to 4.7×10^{-9} ug. eq. for each of the several categories. Each category was run in groups of three animals at least twice. The infusions were made over a period of 15 minutes. An additional 30 minutes was allotted to allow ample time for the extract to stimulate FM in the tooth. The animal was decapitated, the jawbone with the teeth was removed from the mouth, frozen on dry ice, and coded. The frozen teeth were sectioned (Steinman, et. al., 1959) and viewed microscopically under ultraviolet light (Steinman, 1967). The dentin under the occlusal grooves was observed and evidence of the presence of the fluorescent dye, acriflavine hydrochloride, demonstrated by the brilliant green illumination of the odontoblastic processes constituted

evidence of fluid movement in the tooth. A quantitative ratio was expressed using the following formula:

$$\frac{\text{Fluorescing Occlusal Grooves}}{\text{Fluorescing + Nonfluorescing Grooves}} = \text{Fluid Movement Ratio}$$

(Steinman, R. R., and Leonora, J., 1971).

Controls were run using the same procedure and infusing 0.5 ml. of normal saline. Statistical analysis of the data included student's t-test to compare the significance of each dose response to the saline only control response. Correlation and regression analysis was determined for each group to obtain the coefficient of correlation and the significance of each plot.

Chapter 3

RESULTS

Figure 1 shows the stimulatory effects of extracts from the hypothalami of animals maintained on a Purina-fed diet. Purina 0-caries demonstrated its most effective range between 0.0012 ug. eq. and 0.01 ug. eq., the 1-5 caries group between 0.0025 ug. eq. and 0.01 ug. eq. The 6 and above caries group did not demonstrate any significant increase in FM at the doses tested (Figure 1 and Table 3). Table 2 shows that the only significant linear correlation between dose administered and FM response was in the Purina 0-caries group. The Purina 1-5 caries group was able to stimulate FM at higher doses (Table 3), however, significant linear correlation could not be established. The nearly horizontal straight line plot of the 6 and above caries group indicates FM stimulating effect similar to that observed in the saline infused controls.

In Figure 2 relationships between the HSD hypothalamic extracts and FM are shown. Seven groupings demonstrated a pattern of optimal dose levels that could be used to express a relationship between caries present and FM response in the tooth. The HSD-0 caries plot shows that the optimal response range was from 0.0012 ug. eq. to 0.01 ug. eq., the 1-10 caries group between 0.0015 ug. eq. and 0.02 ug. eq., 11-20 caries group between 0.0003 ug. eq. and 0.0012 ug. eq., the 21-30 caries group between 0.0003 ug. eq. and 0.0025 ug. eq., the last group to demonstrate a definitive response was the 31-40 caries group which

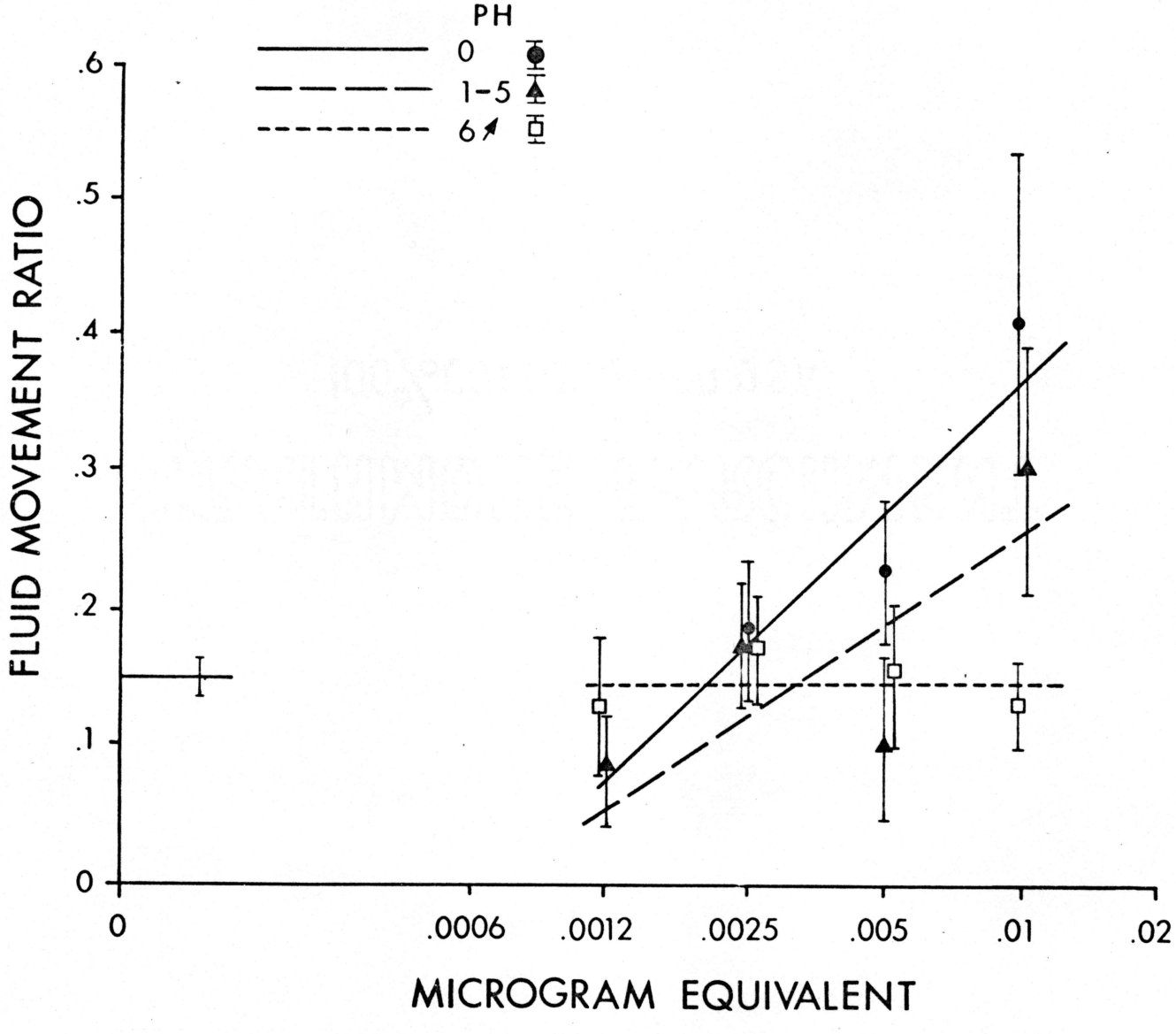


Figure 1. The Bioassay Response of Various Cariogenically Grouped Hypothalamic Extracts From Purina-fed Rats Infused Through the Right Subclavian Vein. Each Point Represents an Average of Six Animals.

Table 2. Linear Correlation and Regression Analysis for the Various Caries Groups

Substance	Linear Correlation Coefficient (r)	Level of Significance ( )
Hypothalamus		
Purina Caries		
0	0.639	0.002
1-5	0.300	-----
6 & Above	0.006	-----
HSD Caries		
0	0.705	0.001
1-10	0.588	0.003
11-20	0.673	0.002
21-30	0.668	0.001
31-40	0.739	0.001
41-50	0.183	-----
51 & Above	0.086	-----
Cortex		
Purina Caries		
0	0.627	0.005
HSD Caries		
0	0.325	-----
51 & Above	0.295	-----

Table 3. Student's t-test Calculation for Each of the Doses Used for Plotting Dose vs. FM Response on Graphs

Substance Infused	Dose ug. eq.	FM Response	Level of Significance
Saline Control	-----	0.154 ± 0.015	-----
Hypothalamus			
Purina Caries			
0	0.0012	0.083 ± 0.045	-----
	0.0025	0.189 ± 0.046	-----
	0.005	0.229 ± 0.050	-----
	0.01	0.418 ± 0.120	0.002
1-5	0.0012	0.255 ± 0.565	-----
	0.0025	0.172 ± 0.053	-----
	0.005	0.109 ± 0.061	-----
	0.01	0.302 ± 0.092	0.01
6 & Above	0.0012	0.132 ± 0.051	-----
	0.0025	0.171 ± 0.040	-----
	0.005	0.156 ± 0.048	-----
	0.01	0.138 ± 0.030	-----
HSD Caries			
0	0.0012	0.098 ± 0.042	-----
	0.0025	0.239 ± 0.069	-----
	0.005	0.303 ± 0.046	0.01
	0.01	0.456 ± 0.029	0.001
1-10	0.0025	0.116 ± 0.047	-----
	0.005	0.167 ± 0.056	-----
	0.001	0.301 ± 0.042	0.01
	0.02	0.333 ± 0.071	0.003
11-20	0.0003	0.104 ± 0.045	-----
	0.0006	0.256 ± 0.069	-----
	0.0012	0.358 ± 0.032	0.001
21-30	0.0003	0.109 ± 0.033	-----
	0.0006	0.154 ± 0.045	-----
	0.0012	0.332 ± 0.046	0.002
	0.0025	0.332 ± 0.044	0.005
31-40	0.0003	0.044 ± 0.016	0.007
	0.0006	0.067 ± 0.030	-----
	0.0012	0.179 ± 0.025	-----
	0.0025	0.241 ± 0.060	-----
	0.005	0.389 ± 0.058	0.001
41-50	0.0025	0.193 ± 0.072	-----
	0.005	0.209 ± 0.026	-----
	0.01	0.266 ± 0.048	-----
	0.02	0.161 ± 0.037	-----
	0.03	0.316 ± 0.081	0.007

Table 3 continued

Substance Infused	Dose ug. eq.	FM Response	Level of Significance
51 & Above	0.0025	0.253 ± 0.059	-----
	0.005	0.260 ± 0.066	-----
	0.01	0.128 ± 0.035	-----
	0.02	0.277 ± 0.073	0.038
	0.03	0.237 ± 0.036	-----
	0.04	0.301 ± 0.059	0.01
Cortex			
Purina Caries			
0	0.0003	0.098 ± 0.028	-----
	0.0006	0.108 ± 0.050	-----
	0.0012	0.355 ± 0.071	0.001
HSD Caries			
0	0.0003	0.136 ± 0.021	-----
	0.0006	0.200 ± 0.059	-----
	0.0012	0.217 ± 0.041	-----
	0.0025	0.215 ± 0.026	-----
51 & Above	0.0003	0.178 ± 0.036	-----
	0.0006	0.156 ± 0.055	-----
	0.0012	0.234 ± 0.092	-----
	0.0025	0.221 ± 0.048	-----
	0.005	0.296 ± 0.028	0.01

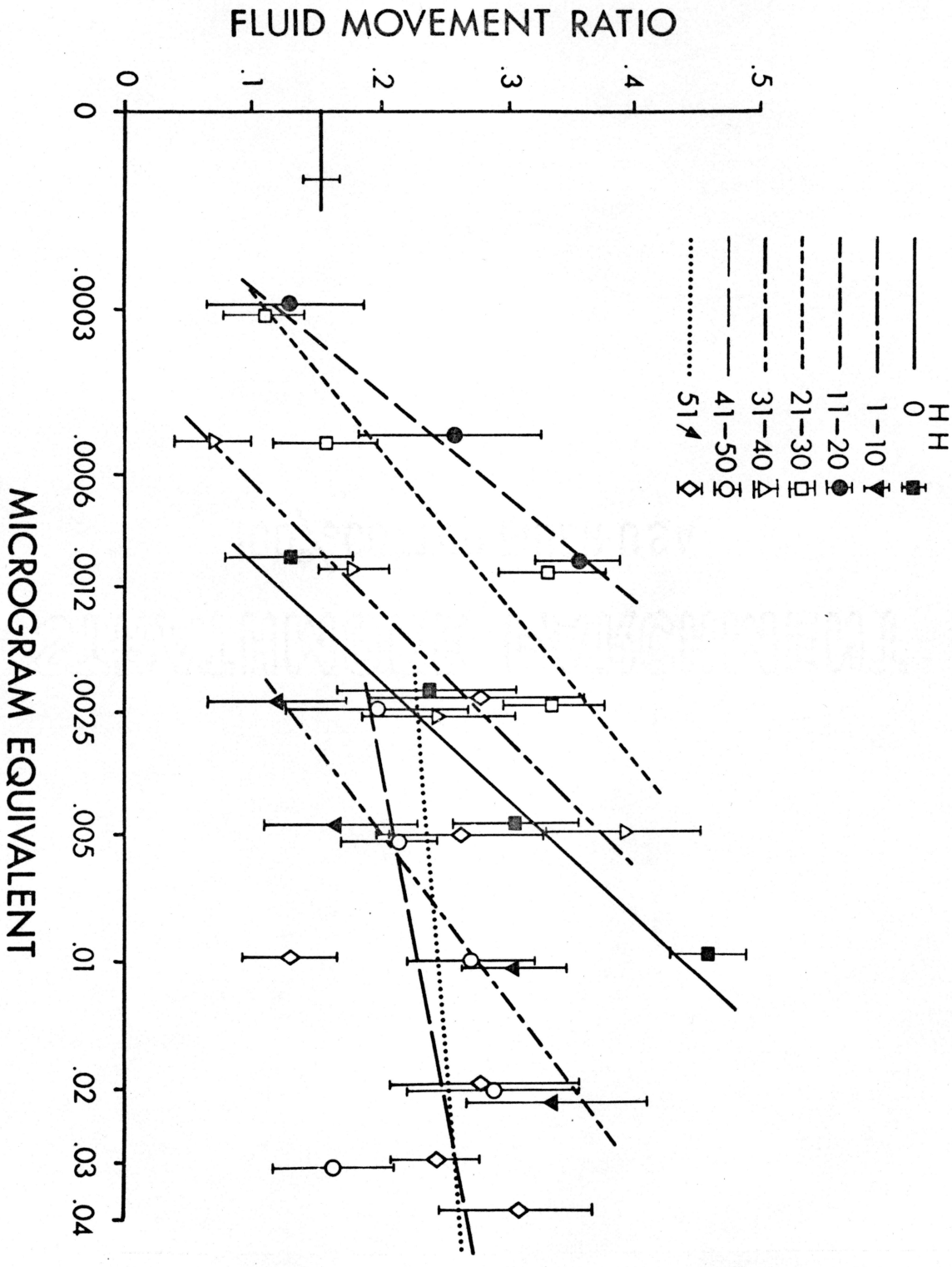


Figure 2. The Bioassay Response of Various Cariogenically Grouped Cariogenically Grouped Hypothalamic Extracts From High Sugar Diet-fed Rats Infused Through the Right Subclavian Vein. Each Point Represents an Average of Six Animals.

ranged between 0.0006 ug. eq. and 0.005 ug. eq. In each of the aforementioned groups the highest dose showed a highly significant increase in FM over the saline infused controls (Table 3). In contrast, the two remaining groups, 41-50 and 51 and above caries, did not show any significant increase in the slopes of their dose response curves.

In the hypothalamic extracts from the HSD-fed animals the greatest degree of demonstrated FM stimulation was exhibited by the 11-20 caries group with the 21-30 and the 31-40 caries group showing a gradual decline. The 1-10 caries group displayed less ability to stimulate FM than the HSD-0 caries group or any of the other three groups mentioned.

When the graphs are viewed in light of Table 2 it becomes evident that there is no linear correlation between the 41-50 and 51 and above caries group and FM in the tooth. Figure 2 and Table 2 established the positive linear correlation between the HSD group with caries scores from 0-40 and FM.

Figure 3 graphs the cortical stimulatory abilities of Purina 0-caries, HSD 0-caries, and HSD 51 and above caries. The only statistically significant linear correlation was with the cortical material from the Purina-fed animals when one views the graphs in conjunction with Table 2.

Figure 4 compares the relative stimulatory effects of the 0-caries cortical and hypothalamic extracts from Purina-fed and HSD animals. The indications are that the cortical extracts from the Purina-fed rats are nearly five times as potent as the hypothalamic counterpart.

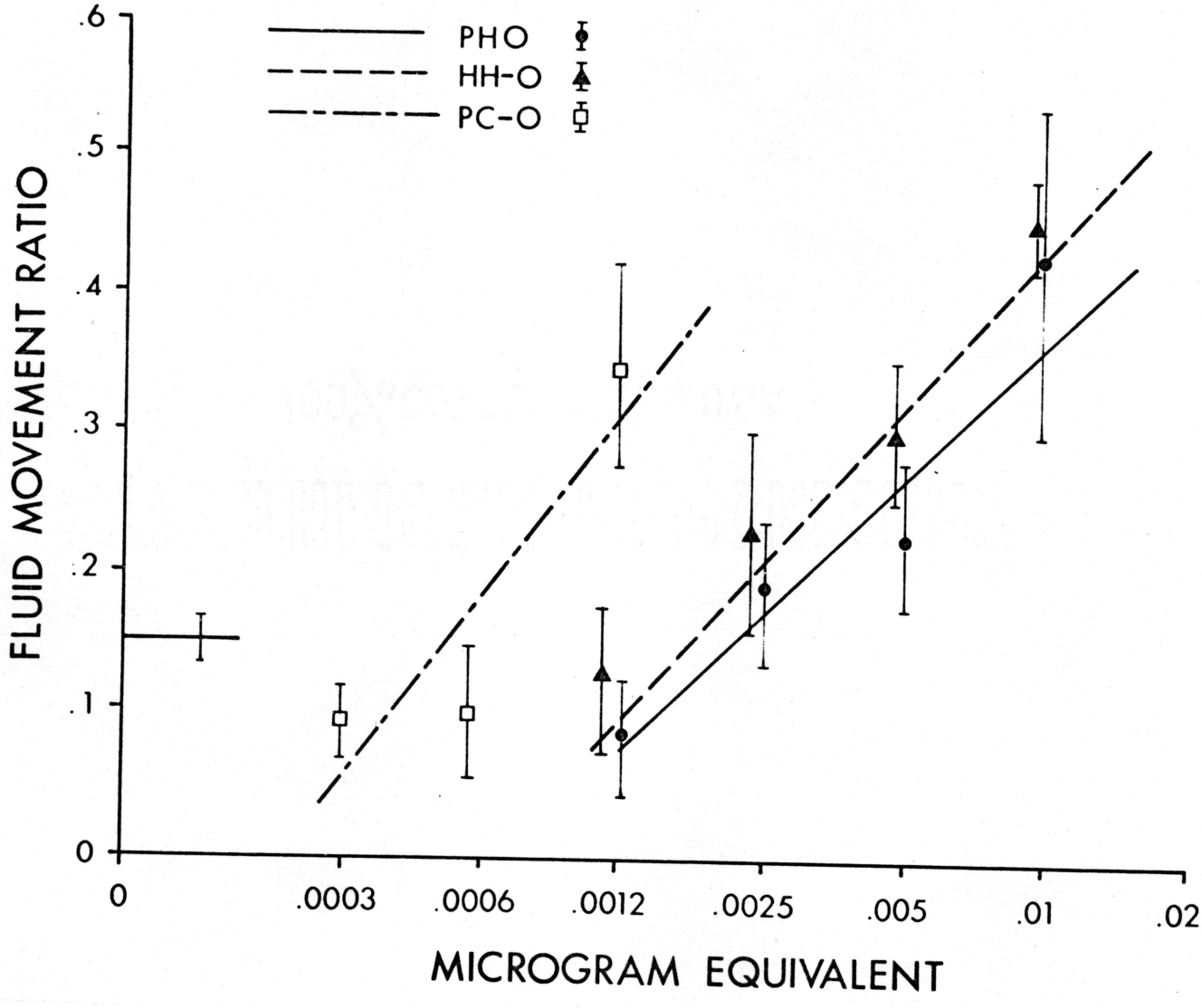


Figure 3. The Bioassay Response of Various Cariogenically Grouped Cortical Extracts From Selected Purina-fed and High Sugar Diet-fed Rats Infused Through the Right Subclavian Vein. Each Point Represents an Average of Six Animals.

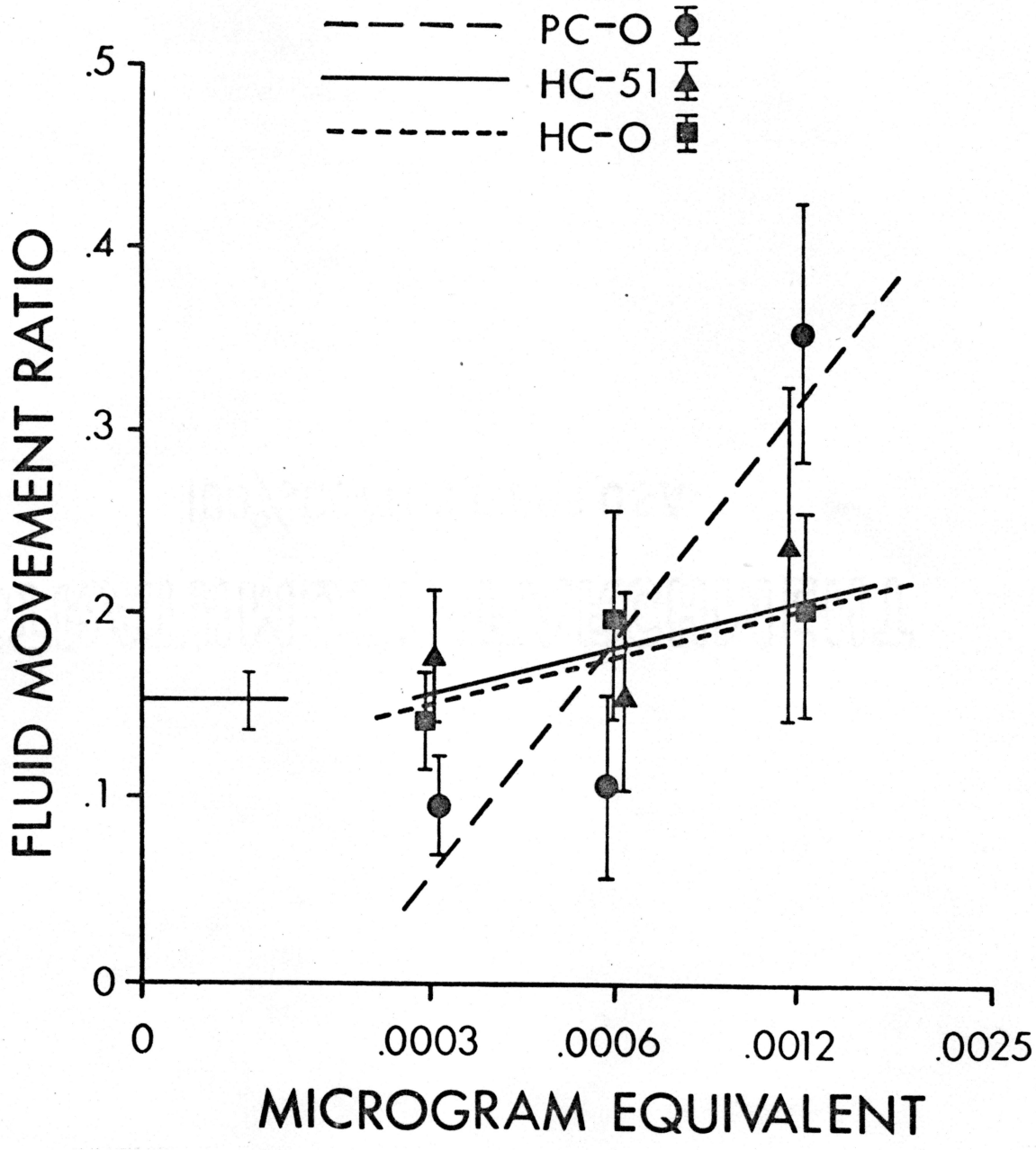


Figure 4. A Comparison of the Bioassay Response of the 0-caries Hypothalamic Extracts of Purina-fed and High Sugar Diet-fed Rats and the 0-caries Cortex of the Purina-fed Rats. Each Point Represents an Average of Six Animals.

Chapter 4

DISCUSSION

Leonora and Steinman (1968) proposed the existence of a hypothalamic parotid endocrine axis (HPEA). In a subsequent article Steinman and Leonora (1975) showed that the cariogenicity of a high sugar diet (HSD) could be reduced to a level equal to or less than that obtained with Purina Lab Chow, a noncariogenic diet. These investigators assumed that the increase in dental caries resulted from a suppression of the function induced by the HPEA. They showed that these suppressive effects could be overcome by compounds, e.g. carbamyl phosphate, which enhanced the fluid movement (FM) mechanism of the HPEA or certain additives: Cr, Mn, Zn, Mo, which facilitate glucose metabolism.

Our initial hypothesis assumed that rats maintained on a Purina Lab Chow diet and displaying no caries demonstrated the optimal homeostatic state for FM since a lack of cavities suggested an intact fluid movement system, and an increase in caries was associated with a decrease in FM resulting from a breakdown in the HPEA. The Purina-fed animals with 0-caries demonstrated a correlation between hypothalamic and cortical functions not evident in any of the other tested groups, both hypothalamic and cortical extracts used as infusion materials were able to significantly stimulate FM in the tooth. These results indicate a relationship between optimal hypothalamic function and cortical activity. The superior stimulating ability of the cortical extract

indicate that it is somehow integrally involved, perhaps with the recycling of hypothalamic secretions in excess of metabolic need, in the normal homeostatic mechanism, or that the parotid-hormone releasing factor (PRF) is synthesized at extra-hypothalamic centers. Recent researchers have discussed the presence of releasing factors in brain material other than the hypothalamus (Zimmerman, 1976).

As the number of caries increased in the Purina-fed rats the hypothalamic extracts gave no indication of the factor being concentrated, but relative impotency was quickly reached, as demonstrated by its diminished ability to enhance FM above the level of the saline infused control animals. It is not known whether impotency was due to some failing of the hypothalamus, that is, the quality of the PRF produced was inferior, the quantity of the factor insufficient, or the ratio of synthesis to secretion was no longer physiologically optimal. Perhaps the seat of the problem was the parotid gland, the end organ itself, or any of a number of probable intermediary sites capable of becoming refractory.

Our study of the HSD-fed animals indicates that the hypothalamus, when presented with the challenge to maintain optimal FM, is stimulated to produce a hypothalamic parotid hormone releasing factor that increases in potency as the number of caries increase to a given point, and finally decompensates when it is not able to facilitate the desired response in the end organ. The 1-10 caries group was approximately one-third as potent as the 0-caries group but had definite stimulating ability, the 11-20 caries group was about seven times as

potent, the 21-30 caries group about three times as potent and the 31-40 caries group approximately twice as potent. The two remaining groups, 41-50 and 51 and above caries, lacked stimulating ability at the tested levels. These findings suggest that the inferior potency of the extract from the 1-10 caries group indicates that activation of the appropriate mechanism is perhaps not as dramatic as when the caries score increases, or that the normal release of the factor was somehow being altered. It should be noted that the average number of caries in this group was nearer zero than 10 (Table 4).

An interesting comparison can be drawn between the responses of the Purina-fed caries group 1-5 and the HSD-fed 1-10 caries group. Table 4 shows that the mean for the Purina-fed rats and the mean for the HSD-fed rats were relatively comparable. The graphed responses are also comparable with both being about one third as potent as their 0-caries counterpart. This suggests that extracts derived from animals with low caries scores do not trigger an adequate response in the hypothalamus to maintain the degree of potency of the physiologically successful, 0-caries group.

As the caries score increases to 11-20 the hypothalamus appears to be maximally stimulated to produce a factor sufficiently more potent than the 0-caries group, to suggest that it is even more capable of preventing tooth decay by presenting a stronger stimulus to FM. It appears that the negative feedback control of the hypothalamus has been impaired and that the hypothalamus respond by increasing its synthesis and/or secretion of parotid hormone releasing factor.

Table 4. Mean Caries Scores and Sample Size for Various Caries Group

Group	Mean Caries Score	Sample Size
Purina-fed		
0	0	22
1-5	2.2 ± 0.4	12
6 & Above	9.7 ± 1.4	9
HSD-fed		
0	0	76
1-10	4.4 ± 0.4	58
11-20	16.4 ± 0.6	19
21-30	25.7 ± 0.6	22
31-40	34.0 ± 0.7	13
41-50	44.4 ± 0.3	11
51 & Above	62.0 ± 3.1	6

Caries scores beyond 20 show the titer of PRF decreasing, suggesting that the hypothalamus is gradually exhausting its synthesizing ability. Since high concentrations of PRF were unable to counteract the attenuated FM in the tooth and halt the decay process, a likely probability is that some point or points in the HPEA have become refractory to its cues. The caries groups with scores above 41 may be indicative of a fatigued hypothalamus, no longer capable of compensating for a failing system, or these groups may demonstrate an adversely affected synthesis to secretion ratio.

Chapter 5

CONCLUSION

Marked increases in the FM stimulatory abilities were observed in the Purina 0-caries hypothalamic and cortical extracts above the saline infused control rats. Increased caries in the Purina-fed animals were apparently not at a high enough level to stimulate increased hypothalamic synthesis of the PRF. The synthesis to secretion ratio may have been such that the homogenized hypothalamus did not demonstrate a reserve titer capable of stimulating FM significantly above the levels achieved by infusing saline into the control animals.

The sucrose in the diet of the HSD-fed animals appears to act as a metabolic dictator adversely affecting the normal release of the PRF in the hypothalamus. Low caries in the HSD-fed animals responded much like the increased caries of the Purina-fed animals with a less potent extract being present in the hypothalamus. The 11-20 caries group exhibited the most potent FM stimulating ability with a gradual diminution of potency until the scores reached 40.

Extracts from these hypothalami appear to have the potential to block further caries development. This strongly suggests that synthesis, or the synthesized materials, was not the problem in diminished FM. The tested groups above 40 caries demonstrate a hypothalamus unresponsive to the high level of caries, probably due to the fatiguing of the system in its prior attempts to regain physiological stability.

Even when the hypothalamus is optimally stimulated to produce a more potent extract, the level of tooth decay remains high. From this it may be concluded that if the failing is in the hypothalamus, the release, or the release-to-synthesis ratio is probably the weak link in the chain, rather than synthesis. Some clues to strengthening this weak link may lie in determining the actual cortical contribution in the physiologically optimal state. If the hypothalamus is functioning adequately the problem could lie in a refractory parotid gland or odontoblast.

BIBLIOGRAPHY

Armstrong, W. D. and Barnum, C. P. Concurrent Use of Radioisotopes of Calcium and Phosphorus in the Study of the Metabolism of Calcified Tissue. J. Biol. Chem. 172:199, 1948.

Atkinson, H. F. The permeability of human enamel. Brit. Dent. J. 83:205-214, 1947.

Atkinson, H. F. The permeability of human enamel. Brit. Den. J. 84:113-119, 1948.

Atkinson, H. F. and Parker, D. A. S. Bioelectric properties of the tooth. J. Dent. Res. 48:789-794, 1969.

Bergman, G. and Linden, L. Techniques for microscopic study of the enamel fluid in vivo. J. Dent. Res. 44:1490, 1965.

Beust, T. B. von. A contribution to the study of immunity to dental caries. Dent. Cosmos. 54:659-663, 1912.

Beust, T. B. von. Reactions of the dentinal fibril to external radiation. J. A. Dent. Assoc. 18:1060, 1931.

"Blakiston's New Gould Medical Dictionary." McGraw Hill, New York, 1956. Cited from Art and Science of Dental Caries Research, ed. Harris, R. S. New York: Academic Press, 1968.

Bodecker, C. F. Nutrition of the enamel of the human teeth. Dent. Cosmos. 53:1000-1011, 1911.

Bodecker, C. F. Permeability of the enamel in relation to stains. J. Natl. Dent. Assoc. 10:10-63, 1923.

Bodecker, C. F. A new theory of the cause of dental caries. Dent. Cosmos. 71:586-598, 1929.

Bodecker, C. F. and Applebaum, E. Metabolism of the dentin; its relation to dental caries and to the treatment of sensitive teeth. Dent. Cosmos. 73:995, 1931.

Bodecker, C. F. and Solewen, L. The influence of unilateral dental caries in 516 cases. J. Dent. Res. 16:401, 1937.

Bodecker, C. F. and Lefkowitz, W. Further observations on vital staining of dentin and enamel. J. Dent. Res. 28:387, 1946.

Brannstrom, M. and Linda, P. O. Pulpal response to early dental caries. J. Dent. Res. 44:1045, 1965.

"Dorland's Illustrated Medical Dictionary." Saunders, Philadelphia, Penn., 1965. Cited from Art and Science of Dental Caries Research, ed. Harris, R. S. New York: Academic Press, 1968.

Fish, E. W. Circulation of lymph in the dentinal tubules, with some observations on the metabolism of the dentine. Proc. Royal Soc. Med. 19:59-72, 1926.

Fish, E. W. The lymph supply of the dentine and enamel. Brit. Dent. J. 48:149-157, 1927a.

Fish, E. W. The circulation of lymph in dentine and enamel. J. Amer. Dent. Assoc. 14:804-817, 1927b.

Gibbons, R. J. Bacteriology of dental caries. J. Dent. Res. 43:1021, 1964.

Gibbons, R. J. Art and Science of Dental Caries Research, ed. Harris, R. S. New York: Academic Press, 1968.

Gwinnett, A. J. A comparison of proximal carious lesions as seen by clinical radiography, contact microradiography, and light microscopy. J. Amer. Dent. Assoc.

Keyes, P. H. Art and Science of Dental Caries Research, ed. Harris, R. S. New York: Academic Press, 1968.

"
Kolliker, A. Manual of human microscopal anatomy, Philadelphaa, 1854. Cited from Kreudenstein, S. von.

Kreudenstein, S. von. Dentinstoffwechselstudein-I. Mitteilung. Ube den dentinliquor. Dtsch. Zahnarztl, Z. 10:473-476, 1955.

Kreudenstein, S. von and Stueben, J. Dentinstoffwechselstudein-III. Mitteilung. Die thermische methode zum nackweis des dentin-lequors. Dtsch. Zahnarztl, Z. 10:1178-1182, 1955.

Kreudenstein, S. von, and Stueben, J. Dentinsroffwechselstudein-IV. Mitteilung. Uber die gewinnung des dentinlequors durch elution zum zwick der bestimming seiner bestandteile sowie untersuchungen uber den wassergeholt des dentins und uber des dentinlequor spatium. Dtsch. Zaharztl, Z. 11:1214-1220.

Lefkowitz, W. Further observations of dental lymph in the dentine. J. Dent. Res. 22:287-292, 1943.

Leonora, J. and Steinman, R. R. Evidence suggesting the existence of a hypothalamic-parotid gland endocrine axis. Endocrinology 83:807, 1968.

Pincus, P. Passage of phosphate through the human tooth. Nature. 18:844-845, 1958.

Scherp, H. W. Dental caries: prospects for prevention. Science. 173:1199-1205, 1971.

Sharpenak, A. E. The problem of the prevention of dental caries. Review of Stomatologie, (Paris) 73:643, 1972.

Socransky, S. S. The use of saliva and plaque tests in evaluating anti-caries therapeutic agents. Proc. of the Conf. on the Clinical Testing of Cariostatic Agents NIH, 1972.

Sognnaes, R. F., Shaw, J. H., and Bogozoch, R. Radiotracer studies on bone, cementum, dentin, and enamel of Rhesus monkeys. Amer. J. Physiol. 180:408-420.

Sanchez, A., Steinman, R. R., Leonora, J. Effects of diet on dentinal fluid movement in germfree and nongermfree rats. A. J. Clinic. Nutr. 23:686-688, 1970.

"Stedman's Medical Dictionary." Williams & Wilkins, Baltimore, Maryland, 1961. Cited from Art and Science of Dental Caries Research, ed. Harris, R. S. New York: Academic Press, 1968.

Steinman, R. R. Histochemical & radiographic changes observed in human teeth. J. South. Calif. State Dent. Assoc. 29:294-300, 1961.

Steinman, R. R. The movement of acriflavine hydrochloride through the molars of rats on a cariogenic and noncariogenic diet. J. Southern Calif. State Dental Assoc. 35:151-157, 1967.

Steinman, R. R., Hardinge, M. G., Woods, R. W. Experimental caries with human foods. J. Dent. Res. 37:865-873, 1958.

Steinman, R. R., Hewes, C. G., and Woods, R. W. Histochemical analysis of lesions in incipient dental caries. J. Dent. Res. 38:592-605, 1959.

Steinman, R. R. and Leonora, J. Relationship of fluid incidence of dental caries. J. Dent. Res. 50:1536, 1971.

Steinman, R. R. and Leonora, J. Effects of selected dietary additives on the incidence of dental caries in rats. J. Dent. Res. 54:570-577, 1975.

Stueben, J. and Kreudenstein, S. von. Dentinstoffweihselstudein - V. Mitteilung. Papier chromatographische untusuchungen uben den Gehalt anfreien aminosauren im Dentinliquor memschlicher Zäkne. Dtsch. Zahnarztl. Z. 11:1448-1454.

Volker, J. F. and Sognnaes, R. F. A study of the phosphorous metabolism in the dental tissue of the cat by the use of radioactive phosphorous. J. Dent. Res. 19:292, 1940.

Zimmerman, E. A. Frontiers in Neuroendocrinology, vol. 4. ed. Martini, L. and Ganong, W. F. New York: Raven Press, 1976.

Zipkin, I. Art and Science of Dental Caries Research. ed. Harris, R. S. New York: Academic Press, 1968.