Reprinted from ORAL SURGERY, ORAL MEDICINE AND ORAL PATHOLOGY, St. Louis.
Vol. 15, No. 5, Pages 555-565, May, 1962. (Printed in the U. S. A.)

ASCORBIC ACID AND ALVEOLAR BONE LOSS

A. E. Thomas, D.M.D., M. C. Busby, Jr., B.S.,
W. M. Ringsdorf, Jr., D.M.D., M.S., and E. Cheraskin, M.D., D.M.D.,
Birmingham, Ala.

University of Alabama School of Dentistry

INTRODUCTION

There is still considerable discussion regarding the etiological factor or factors in alveolar bone loss. There is suggestive evidence that the less the intake of vitamin C, the greater the alveolar bone loss. This investigation was designed to analyze the relationship of ascorbic acid status and bone height in the human organism in the hope of obtaining more information on this controversial problem.

REVIEW OF THE LITERATURE

In order to appreciate the problem and the method of approach, it is necessary to review (1) ascorbic acid and bone physiology and (2) ascorbic acid and bone pathology.

Ascorbic Acid and Bone Physiology.—There is definite evidence to indicate that bone status at any one moment is directly related to the interplay of factors contributing to *bone formation* and *bone resorption.*[1, 2] If these two mechanisms are operating in equilibrium, then bone is neither gained nor lost. Should bone formation be less than bone resorption, then bone loss can be demonstrated.

Furthermore, investigations have definitely demonstrated that bone formation occurs in two phases—the deposition of an osteoid matrix[3] and its subse-

This investigation was supported in part by a grant (A-2899) from the National Institute of Arthritis and Metabolic Diseases and the Florida Citrus Commission.

Editor's Note: This article should be of special interest to teachers and researchers in the field of oral roentgenology. Although the content of the manuscript is not of the type ordinarily submitted to this section, articles of this nature are both informative and refreshing.

quent mineralization.[4] One of the ingredients necessary for osteoid matrix formation is ascorbic acid.[5,6] Therefore, if there is a deficiency of ascorbic acid, bone formation is delayed and the end result is a reduction in bone.

Ascorbic Acid and Bone Pathology.—Studies of this relationship have been completed in both lower animals and human beings. A number of these investigations[7-19] suggest that ascorbic acid deprivation in lower animals (both young and adult) produces osteoporosis.

Wolbach and co-workers[7-9] assert that the osteoporosis observed in scorbutic guinea pigs is due primarily to the failure of cells to produce intercellular substances. This pathologic sequence of events is reversible with the addition of ascorbic acid. MacLean and his group[11] believe that the chief bone lesion caused by vitamin C deficiency is either in the differentiation of mesenchymal cells to osteoblasts or in the stimulation of the osteoblasts to secrete bone matrix. Koreki[12] demonstrated that vitamin C aids in the control of the rate of phosphorus metabolism in teeth and bones. Scorbutic guinea pigs show a reduction of 47 to 70 per cent in the P^{32} metabolism in the teeth and 51 to 75 per cent in bone. This pattern was reversed when vitamin C was introduced.

Cowan and co-workers,[13] in a study of guinea pigs, concluded that the alveolar bone level closely parallels ascorbic acid dosage and that the alveolar bone protective dosage exceeds all other popular recommended levels. Harman and colleagues[14] likewise agree that the integrity of alveolar bone is not maintained in vitamin C-deficient guinea pigs.

Boyle and his co-investigators[15-17] conclude that in both acute and chronic scurvy a generalized alveolar bone rarefaction occurs as a result of the failure of osteoblasts to produce bone matrix and the failure of fibroblasts to form collagen. They also showed extensive osteoclastic activity. These pathologic changes resulted in clinical mobility of the teeth. Other investigations utilizing guinea pigs[18,19] and monkeys[20] confirm these findings and emphasize the increased osteoclasia.

Robertson and Schwartz[21] maintained that the role of ascorbic acid in collagen formation is in the transformation of precollagen to collagen. On the other hand, there is a decided disagreement among researchers concerning the relationship between vitamin C and periodontal disturbances in human studies.

Burrill[22,23] and Radusch[24] conclude that there is little or no relationship between ascorbic acid and periodontal pathosis. However, it should be noted that Burrill studied relatively young persons (medical students) for a short period of time (seven months).

Another group of investigators contend that there is an inverse relationship between periodontoclasia and plasma ascorbic acid levels. Weisberger and co-workers[25] observed that tooth mobility and periodontal pocket formation in patients with low blood levels of vitamin C were present only in the subjects over 20 years of age. Blockley and Baenziger[26] demonstrated that persons with normal gingiva showed a mean vitamin C blood level of 0.76 mg. per cent. Persons with inflamed gingiva demonstrated a mean plasma ascorbic acid level of 0.32 mg. per cent, and others with highly inflamed gingiva had an average blood level of 0.08 mg. per cent.

Thomas,[27] in a clinical study of fifty-four dental students, suggested that the group which received an increased daily intake of orange juice had less gingival pathosis and alveolar bone loss than a control group who were not provided with supplementation.

In this review of the literature a report dealing specifically with the relationship between plasma ascorbic acid levels and alveolar bone loss or bone height was not found.

METHOD OF INVESTIGATION

In this investigation of the relationship of ascorbic acid to alveolar bone loss, fifty-four dental students (mean age, 27 years) were selected on a voluntary basis.[27] Before any examinations were made, the students were divided into an experimental group and a control group of equal numbers on a completely arbitrary basis by means of self-election. Each student was then given a complete oral examination. The condition of the gingiva was especially noted. Histories of the smoking habits of the students were recorded, and a full-mouth roentgenographic survey was also made of each student.

Following collection of the initial data and throughout the subsequent twelve-month period, each participant in the experimental group (to be referred to hereafter as the *supplemented* group) was instructed to drink 8 ounces of a standardized orange juice each morning before breakfast. The orange juice was issued each week in a concentrated form and diluted to specifications[27] by the student. The participants were supervised carefully to ensure cooperation in the procedure. Except for the additional intake of the 8 ounces of orange juice per day, there was to be no change in the normal daily diet of each participant.

In contrast to the supplemented group, the control group (to be referred to hereafter as the *nonsupplemented* group) was instructed specifically to refrain from consuming citrus fruit in order to validate the experimental procedure. This was re-emphasized at frequent intervals during the subsequent experimental year. Except for the elimination of citrus fruits in any form from the diet, no attempt was made to alter the normal daily dietary habits of this group. At the end of the experimental period (twelve months) each dental student was recalled and submitted to a re-examination of the oral structures, both clinically and roentgenographically, as in the initial procedures.

An impartial examiner was selected to perform the measurements of alveolar bone loss. The measurements[28] were made with a specially constructed transparent ruler on which ten radii were marked, each equidistant from the other (Fig. 1). The first radius was placed exactly 1 mm. below the upper margin of the plastic strip, thus allowing for the so-called normal distance of the alveolar crest to the cementoenamel junction. To obtain the measurement, the roentgenograms were placed on an illuminated board. The ruler was positioned on top of the roentgenograms, so that the upper margin coincided with the interproximal cementoenamel junction and the last radius was superimposed over the apex of the tooth being measured. This procedure allowed the examiner to observe the height of the alveolar crest (in percentage) through the ruler and to determine whether the alveolar crests were on or between two radii. Thus, measurements were obtained to the nearest 5 per cent.

Measurements were obtained in this manner for both the mesial and the distal interproximal surfaces of the lower central and lateral incisors. This area was chosen because (1) all subjects possessed lower anterior teeth and (2) roentgenographic distortion is minimized. Those teeth which could not be measured accurately because of rotation, interproximal caries and restorations, and indefinite apices were excluded. Roentgenograms were eliminated from

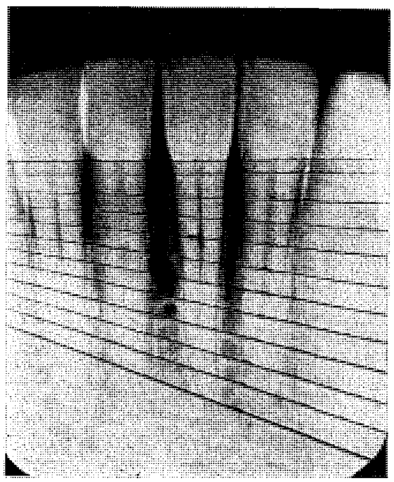

Fig. 1.—Method of measuring alveolar bone loss.

the study if, because of vertical or horizontal angulation, the teeth could not be measured accurately and if there was evidence of cervical burnout. Because of these, one participant in the nonsupplemented group was excluded from the analysis.

RESULTS

The original data derived from the measurement of alveolar bone height (in percentages) of the fifty-three dental students are provided in Table I. The mean values have been pictorialized in Fig. 2. Examination of that chart reveals that at the start of the study there was a slight (0.6 per cent) difference in average alveolar bone heights of the two groups. Further study of Fig. 2 reveals that the group without vitamin supplementation decreased 1.5 per cent during the one-year period (93.8 to 92.3), whereas the supplemented group declined 1.2 per cent during the same period (94.4 to 93.2). Thus, superficially, it appeared that there was a slightly greater alveolar bone loss (0.3 per cent) in the nonsupplemented group than in the supplemented groups. Fig. 3 provides this same information on an absolute basis and emphasizes more vividly the changes of the two groups during the twelve-month period.

TABLE I. MEAN PERCENTAGE ALVEOLAR BONE HEIGHT VALUES

	NONSUPPLEMENTED GROUP		SUPPLEMENTED GROUP	
	START OF EXPERIMENT	END OF EXPERIMENT	START OF EXPERIMENT	END OF EXPERIMENT
	99	98	100	97
	99	98	98	99
	98	99	98	99
	98	96	98	98
	98	95	98	97
	98	95	98	96
	98	94	98	96
	97	97	97	99
	97	96	97	96
	97	94	97	95
	96	96	96	95
	96	95	96	95
	96	95	96	94
	94	93	96	93
	94	93	95	93
	94	89	94	95
	93	92	94	93
	93	92	94	93
	93	92	94	92
	93	89	92	93
	92	92	92	91
	90	83	91	90
	89	90	89	86
	89	88	89	85
	85	83	88	83
	74	76	87	87
			86	92
Mean	93.8	92.3	94.4	93.2
Standard deviation	5.4	5.3	3.9	4.1

All of the possible combinations have been studied statistically (Table II) by means of the student "t" test. It is clear from this chart that not one of the comparisons is statistically significant. For example, the 0.6 per cent difference in bone height between the supplemented and nonsupplemented groups at the start of the study yielded a P value of .500. A statistical evaluation of the bone height of the two groups at the end of the one-year period shows that there is no statistically significant difference (.500). Finally, the values for the two groups at the start and at the end of the experiment also yield no statistically significant difference ($P > .2$).

TABLE II. PROBABILITY VALUES FOR THE VARIOUS GROUPS

	FINAL NONSUPPLEMETED	INITIAL SUPPLEMENTED	FINAL SUPPLEMENTED
Initial nonsupplemented	> .200	.500	
Final nonsupplemented			.500
Initial supplemented			> .200

Thus, within the limits of these data, one might conclude that supplementation with vitamin C did not appreciably alter the alveolar bone height in these groups of dental students.

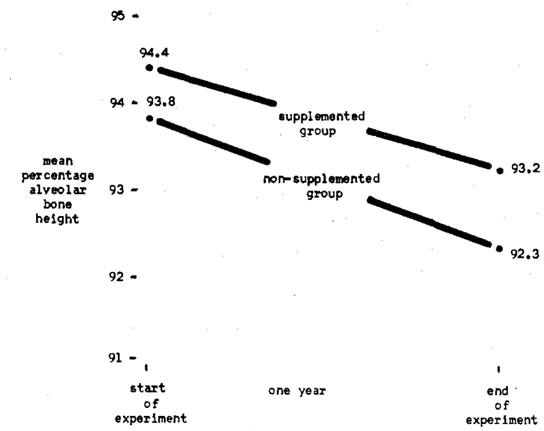

Fig. 2.—Mean percentage alveolar bone height in supplemented and nonsupplemented groups at start and end of study.

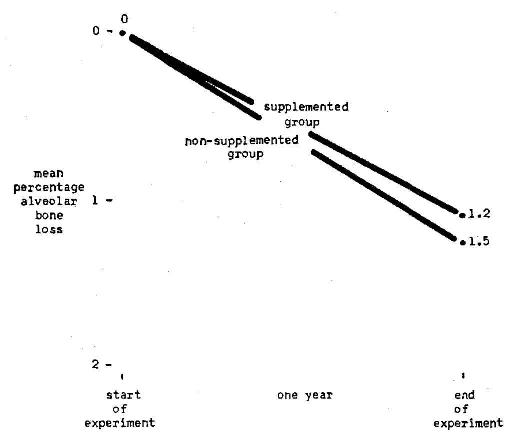

Fig. 3.—Mean percentage alveolar bone loss in supplemented and nonsupplemented groups at start and end of study.

DISCUSSION

It is evident that, from the available data, one cannot conclude any cause-and-effect relationship between ascorbic acid intake and alveolar bone loss. It is possible that such a relationship does not exist. On the other hand, there is the possibility that the samples were too small for true differences to be detected. Also, there is the possibility that the time factor may be the variable which has not been given sufficient consideration. In other words, it is conceivable that there does, indeed, exist a relationship between ascorbic acid and alveolar bone loss but that a period of more than one year is required to demonstrate evidence of this relationship. This possibility indicates the need for a longitudinal study. Such an investigation was not carried out. However, it was thought interesting to extrapolate and see what the alveolar bone height

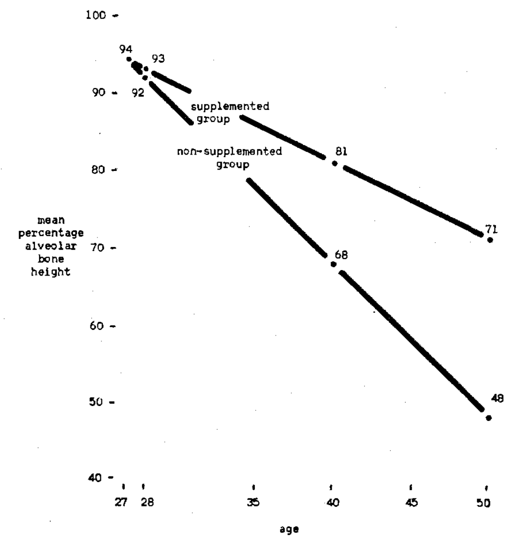

Fig. 4.—Mean percentage alveolar bone height extrapolations for nonsupplemented and supplemented groups.

patterns would be for these 27-year-old students if they were to continue as they had for the one-year period.

Fig. 4 shows the findings at the start of the experiment. In whole numbers, both the supplemented and nonsupplemented groups showed a mean percentage alveolar bone loss of 6 per cent (alveolar bone height, 94 per cent). One year later, the supplemented group lost 1 per cent in alveolar bone height (from 94 to 93 per cent), while alveolar bone height in the nonsupplemented group was reduced by approximately 2 per cent (from 94 to 92 per cent). In Fig. 4 these lines have been extended to age 50. Since no other information was available, it was assumed that the patterns would continue in a linear fashion. This assumption may be questioned. However, assuming such a pattern to be correct, one might estimate that at age 40 the supplemented group would have approximately 81 per cent alveolar bone height in contrast to approximately 68 per cent for the nonsupplemented group. In other words, there might conceivably be a difference, at age 40, of 13 per cent more alveolar bone loss in the nonsupplemented group than in the supplemented group. Extending these extrapolations to age 50, one notes that the supplemented group might have lost approximately 29 per cent of their alveolar bone, whereas the nonsupplemented group might now be without more than one-half of their alveolar bone (52 per cent).

It should be recalled that the lines extending beyond age 28 in Fig. 4 are pure extrapolations. There is no justification for such extensions from the data in this study. However, we thought that it would be interesting to see if any comparisons could be drawn between these extrapolations and the situation which prevails in the population. Accordingly, forty-six male subjects (in the 25- to 54-year age group) were selected from the patients who visited the

University of Alabama Dental Clinic. Actually, thirty-three subjects, with a mean age of 35 years, were measured as in this experiment. It was found that the mean percentage alveolar bone height was 78 per cent. This value has been plotted in Fig. 5. It is noteworthy that the extrapolated value for the dental students at age 35 and the findings in a group of a dental clinic population of the same age are similar. A similar estimate was made at age 40. Again, the difference between the extrapolations of the data for the dental students and the actual findings of the selected population was only 5 per cent. Finally, a similar comparison at age 45 revealed that the two values varied by only 1 per cent. These observations are not intended to replace a much-needed longitudinal

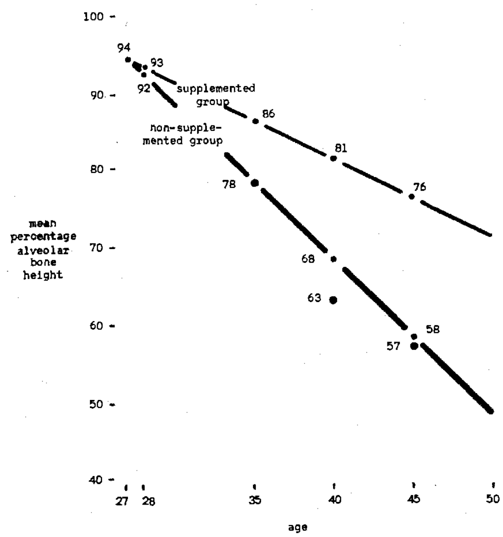

Fig. 5.—Mean percentage alveolar bone height extrapolations for nonsupplemented and supplemented groups.

study. However, they seem to indicate (1) that the extrapolated line may be more real than imaginary and (2) that young dental students without vitamin supplementation might sometime later (with regard to alveolar bone loss, at least) be somewhat similar to the patients who were used to establish estimates of alveolar bone height in later years.

Unfortunately, there are no studies to which one can refer to verify the extrapolated line for those students who received vitamin C supplementation. However, information is available in the laboratory of the Section on Oral Medicine, University of Alabama School of Dentistry, regarding plasma ascorbic acid levels and alveolar bone height in a group of 170 dental patients.[29] Those of the 170 subjects with plasma ascorbic acid levels greater than 0.9 mg. per cent (seventy-three persons) were put in one group. This was done because, according to some investigators,[30] this level of vitamin C is well within physiologic limits. The mean alveolar bone height for the subjects at ages 35, 40, and

45 were determined for this group with acceptable plasma levels. It is noteworthy that the scores obtained closely approximate those of the extrapolated line for the dental students who received vitamin C supplementation (Fig. 6). For example, the extrapolated line at age 35 indicates alveolar bone height of 86 per cent. Fig. 6 reveals that the dental patients with plasma levels greater than 0.9 mg. per cent averaged 87 per cent alveolar bone height (a difference of only 1 per cent). Fig. 6 shows the differences to be 3 per cent at age 40 and only 6 per cent at age 45. Thus, on the average, alveolar bone loss in patients with relatively good plasma ascorbic acid levels is quite similar to the extrapolations for dental students under vitamin C-supplemented conditions.

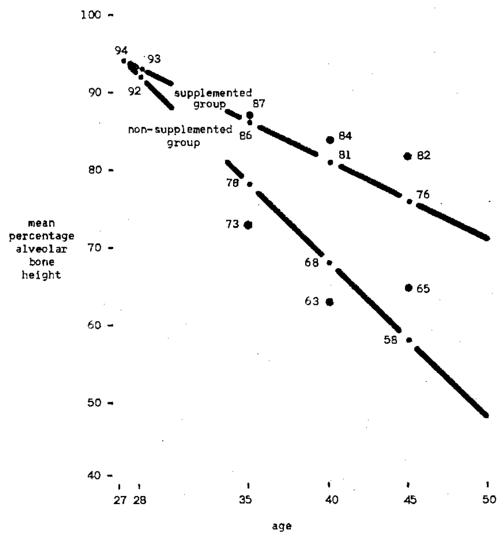

Fig. 6.—Mean percentage alveolar bone height extrapolations for nonsupplemented and supplemented groups.

An attempt was made also to recheck the nonsupplemented group previously discussed in Fig. 4. This recheck was made by studying those of the 170 dental patients (ninety subjects) with plasma ascorbic acid levels less than 0.1 mg. per cent. This level, according to many investigators,[36] is indicative of poor ascorbic acid intake. It can be noted (Fig. 6) that these points closely approximate the extrapolated line for the group of nonsupplemented dental students. For example, at age 35 the prediction for the students is that alveolar bone height will be 78 per cent. The group of dental patients yielded a mean of 73 per cent (a difference of 5 per cent). Likewise, the differences at 40 and 45 years proved to be 5 and 7 per cent, respectively.

SUMMARY

1. This study was designed to evaluate the effect of vitamin C upon alveolar bone height.

2. Fifty-three dental students were observed for a twelve-month period. Half of the students refrained from citrus intake; the other students received frozen orange juice supplementation daily.

3. On a mean basis, the nonsupplemented group lost 0.3 per cent more aleovlar bone than the supplemented group. However, this difference was not statistically significant.

4. Extrapolations of the results of the one-year study were made to age 50. On a mean basis, nonsupplementation yielded 50 per cent alveolar bone loss in contrast to only 25 per cent for the supplemented group.

5. An attempt was made to check the validity of the extrapolations. This check was first made by measuring alveolar bone loss in the same teeth and by the same technique in forty-six patients who visited the clinic for dental care. The mean alveolar bone height values for these subjects at ages 35, 40, and 45 differed from the students at the extrapolated ages of 35, 40, and 45 by 0, 5, and 1 per cent, respectively.

6. A further check was made by plotting alveolar bone height of 35-, 40-, and 45-year-old dental patients with high and low plasma ascorbic acid levels against the students in this study extrapolated to these same ages. The differences ranged from 1 to 7 per cent.

7. It would be highly desirable to compare these findings with those derived from a longitudinal study extending over a period of two to three decades.

The authors wish to acknowledge the editorial assistance of Drs. James W. Clark and Homer Jamison.

REFERENCES

1. Albright, F., and Reifenstein, E. C.: The Parathyroid Glands and Metabolic Bone Disease, Baltimore, 1948, The Williams & Wilkens Company.
2. Cheraskin, E.: Roentgenographic Manifestations of Osseous Changes in the Jaws, ORAL SURG., ORAL MED. & ORAL PATH. 12: 442-458, 1959.
3. McLean, F. C., and Urist, M. R.: Bone, Chicago, 1955, University of Chicago Press.
4. Dallemagne, M. J.: The Physiology of Supporting Tissue, Ann. Rev. Physiol. 12: 101-118, 1950.
5. Shwachman, H., and Gould, B. S.: Blood and Tissue Phosphatase in Experimental Scurvy, Am. J. Dis. Child. 64: 949, 1942.
6. Wolbach, S. B.: The Pathologic Changes Resulting From Vitamin Deficiency, J. A. M. A. 108: 7-13, 1937.
7. Wolbach, S. B., and Howe, P. R.: Intercellular Substances in Experimental Scorbutus, Arch. Path. & Lab. Med. 1: 1-24, 1926.
8. Wolbach, S. B., Howe, P. R., and Church, C. F.: The Pathology of Vitamin Deficiencies, Am. J. Physiol. 90: 560, 1929.
9. Wolbach, S. B., and Bessey, O. A.: Tissue Changes in Vitamin Deficiencies, Physiol. Rev. 22: 233-289, 1942.
10. Christian, P.: Vitamin C (Ascorbic Acid), J. Nair Hosp. D. Col. 2: 35-41, 1950-1951.
11. MacLean, D. L., Sheppard, M., and McHenry, E. W.: Tissue Changes in Ascorbic Acid Deficient Guinea Pigs, Brit. J. Exper. Path. 20: 451-457, 1939.
12. Koreki, S.: Study on P^{32} Metabolism of Teeth and Bones Due to C-Avitaminosis, J. Nihon University School of Dentistry 1: 164-169, 1959.
13. Cowan, R. G., Huffstutler, B. B., Cheraskin, E., and Dunbar, J. B.: Operation Human Guinea Pig, J. Appl. Nutrition 12: 98-116, 1959.
14. Harman, M. T., Kramer, M. M., and Kirgis, H. D.: Lack of Vitamin C in the Diet and Its Effect on the Jaw Bones of Guinea Pigs, J. Nutrition 15: 277-283, 1938.
15. Boyle, P. E., Bessey, O., and Wolbach, S. B.: Experimental Production of Diffuse Alveolar Bone Atrophy Type of Periodontal Disease by Diets Deficient in Ascorbic Acid, J. Am. Dent. A. 24: 1768-1777, 1937.
16. Boyle, P. E.: Dietary Deficiencies as a Factor in the Etiology of Diffuse Alveolar Atrophy, J. Am. Dent. A. 25: 1436-1446, 1938.

17. Boyle, P. E.: Effect of Various Dietary Deficiencies on the Periodontal Tissues of the Guinea Pig and of Man, J. Am. Dent. A. 28: 1788-1793, 1941.
18. Hojer, A., and Westin, G.: Jaws and Teeth in Scorbutic Guinea Pigs, Dental Cosmos 67: 1-24, 1925.
19. Glickman, I.: Acute Vitamin C Deficiency and Periodontal Disease, J. D. Res. 27: 9-23, 1948.
20. Waerhaug, J.: Effect of C-Avitaminosis on the Supporting Structures of the Teeth, J. Periodont. 29: 87-97, 1958.
21. Robertson, W. V. B., and Schwartz, B.: Ascorbic Acid and the Formation of Collagen, J. Biol. Chem. 201: 689-696, 1953.
22. Burrill, D. Y.: Oral Conditions in Experimental Vitamin C and B Deficiency, J. Am. Dent. A. 33: 594-600, 1946.
23. Burrill, D. Y.: Relationship of Blood Plasma Vitamin C Level to Gingival and Periodontal Diseases, J. D. Res. 21: 353-363, 1942.
24. Radusch, D. F.: Relationship Between Periodontal Conditions and Certain Dietary Factors, J. D. Res. 18: 305-327, 1939.
25. Weisberger, D., Young, A. P., and Morse, F. W.: Study of Ascorbic Acid Blood Levels in Dental Patients, J. D. Res. 17: 101-105, 1938.
26. Blockley, C. H., and Baenziger, P. E.: An Investigation Into the Connection Between the Vitamin C Content of the Blood and Periodontal Disturbances, Brit. D. J. 17: 57-62, 1942.
27. Thomas, A. E.: Some Observations on the Influence of Orange Juice Ingestion on the Teeth and Supporting Structures, ORAL SURG., ORAL MED. & ORAL PATH. 7: 741-749, 1954.
28. Schei, O., Waerhaug, J., Lovdal, A., and Arno, A.: Alveolar Bone Loss as Related to Oral Hygiene and Age, J. Periodont, 30: 7-16, 1959.
29. Keller, S. M.: To be published.
30. Cheraskin, E., Dunbar, J. B., and Flynn, F. H.: The Intradermal Ascorbic Acid Test. Part III. A Study of Forty-two Dental Students, J. D. Med. 13: 135-155, 1958.