This is to certify that the

dissertation entitled

SENSORY RESPONSE TO FOOD STIMULI
IN THE ELDERLY AND COLLEGE-age
SUBJECTS

presented by

STEVEN ANTHONY WITHERLY

has been accepted towards fulfillment
of the requirements for

PH.D. degree in FOOD SCIENCE & HUMAN NUTRITION

Rachel Schemmel
Major professor

Date 11-12-82

SENSORY RESPONSE TO FOOD STIMULI IN THE ELDERLY AND COLLEGE-AGE SUBJECTS

By

Steven Anthony Witherly

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Food Science and Human Nutrition

1982

ABSTRACT

SENSORY RESPONSE TO FOOD STIMULI
IN THE ELDERLY AND COLLEGE-AGE
SUBJECTS

By

Steven Anthony Witherly

The purpose of this dissertation was twofold. First, to compare the sensory responses of gustation, olfaction and salivation to food stimuli between two divergent age populations (elderly and college-age subjects) and second, to study the effect of zinc and copper supplementation on the restoration of decreased olfactory and salivation function in a group of eight elderly subjects.

In Study I, a comparison of sensory responses to gustation (saltiness and sweetness, both hedonic and intensity) and olfaction (intensity, odor recognition) was studied in 25 healthy elderly subjects (mean age: 83.0; SD:±5.0) and college students (mean age: 24.0; SD:±7.2). In addition, whole-mouth salivary flow rates were measured in response to sniffing and tasting lemon wedges and lemonade. Utilizing a 17-point, category structured scale, the taste hedonics and intensity responses to 6.0, 8.0, 10.0, 14.0, and 18.0% w/v sucrose in lemonade and 0, 1.0, 2.5, 4.5, and 7.0% NaCl in unsalted tomato juice were studied. Hedonic responses peaked for both groups at the 1.0% w/v NaCl level in tomato juice and the

14.0% w/v sucrose in lemonade. The positively sloped intensity responses to saltiness and sweetness were similar, although the elderly demonstrated a narrower range of response than the college students. In the olfactory studies, subjects compared odor recognition of ten food odorants, including the intensity of the first 7 using an unstructured 100mm line scale. Correct identification of the ten food odors averaged 86% and 33.5% for the college and elderly, respectively. Compared to the elderly, college students rated the seven odorants as being significantly more intense (mean: 61.4 vs. 42, $t = 4.49$, $p < .01$). In the final study, 2-minute salivary flow rates were measured under various conditions. Resting levels of salivary flow were similar for both groups. However, unlike the college students, salivary flow in the elderly did not increase due to sniffing lemons and increased only moderately in response to tasting lemonade.

In Study II, two additional olfactory experiments, utilizing the subjects of Study I, examined whether the elderly would improve their odor recognition scores with practice, and what classes of foods, if any, the elderly might assign to blank sampling cups included with the eight food aromas. As in Study I, the elderly scored below 50% recognition for the majority of the eight food odorants, even when a listing of the foods was placed inside the tastebooth for inspection. Elderly subjects recognized the blank sample cups as blank 50% of the time, with no discernible pattern of food classification in their guessing. In contrast, the college-age subjects approached 100% correct identification for the eight food aromas and two blank sampling cups.

Finally, in Study III, the incorporation of 50 mg of zinc (as zinc citrate), and 5 mg of copper (as an amino acid complex) in the diets of eight elderly for eight weeks had little effect upon the elderly's perception of aroma intensity, olfactory recognition or salivation to food stimuli as compared to the previous baselines established before supplementation. Some subjects, however, noticed an increase in general appetite, while one subject demonstrated a remarkable increase in overall salivation (2-3 times greater).

Analysis of the hair in Study I revealed no significant difference in zinc level between the college (mean: 214.5 $\pm$ 61 µg/g) and elderly (mean: 188.3 $\pm$ 47 µg/g); while copper levels were significant at $p<05$ (39.2 19 vs. 27.5 12 µg/g) between the college and elderly subjects, respectively. In Study II, use of a zinc/copper supplement did not significantly increase hair levels of the same (zinc: 210-223 µg/g; copper: 28-33 µg/g).

In summary, the elderly and college-aged subjects displayed surprisingly similar sensory scaling responses to suprathreshold concentrations of NaCl and sucrose. The elderly, however, possessed greatly decreased olfactory discrimination as measured by percent correct response to food aroma identification. The elderly also demonstrated decreased salivary secretion to gustatory and olfactory food stimuli as compared to their college-age counterparts. It was postulated that the elderly possess the ability to detect the intensity of an odorant without the ability to discriminate among odorants.

ACKNOWLEDGEMENTS

Sincere appreciation is expressed to all subjects in the study, both Michigan State University graduate students and residents of Burcham Hills Retirement Center. The cooperation of Mr. Redheffer, Administrator, Mrs. Nancy Herbert, Dietitian, and other staff at Burcham helped immeasurably in the completion of the study.

The joint effort of Georgene Pijut and Paula Hungerman, MSU undergraduates who assisted with the technical work, made the research a reality. Their efforts typified the high caliber of help I received throughout the completion of the experimental protocols. Mike Dauria is to be commended for his hard work and dedication--everywhere apparent in the completion of the studies and this dissertation.

The research was supported in part by NIH Nutrition Traineeship Grant GM 0810-02 which partially supported my graduate assistantship and a Michigan State University Foundation Grant.

I am also indebted to those kind individuals who saw fit to further fund this project, in particular, Dr. L. J. Minor, president of the Minor Corporation; and the David T. Witherly Engineering Company.

Finally, regards and appreciation are warranted to my fine thesis committee, consisting of Drs. Zabik, Bernard, Luecke and O'Kelly, and my major professor, Dr. Schemmel.

TABLE OF CONTENTS

LIST OF FIGURES

I. INTRODUCTION

The number of older individuals in the United States has increased dramatically in the last 50 years, and demographers are predicting the continuation of this trend. Twenty-million persons over the age of 65 lived in this country in 1970 and projections indicate that the number has increased by 3 to 4 million since then (Anonymous, 1979).

Although the chronic diseases of later life-hypertension, diabetes, heart disease and cancer - have received the bulk of scientific scrutiny, little attention has been focused upon the consequences of increased life span and gustatory or olfactory function.

Therefore, the aim of this present work is to delineate and quantify those age-related changes in sensory preception and salivation by comparing a group of college-aged individuals with a healthy subset of elderly who averaged 83 years of age. In addition, food intakes in the form of a three-day dietary record will be examined and analyzed in an attempt to correlate nutrient intake, especially of zinc and copper, with its relationship to sensory perception. Finally, a group of eight elderly will receive a supplement of zinc and copper for sixty days in order to study the influence of increased mineral nutrition and gustatory, olfactory or salivary restorations.

REFERENCES

Anonymous, "Aging and Nutrition", Ross Laboratories, Columbus, Ohio, P. 3, 1979.

II. LITERATURE REVIEW

I.1 Gustatory and Olfactory Discrimination in the Elderly

Gustatory Discrimination

The effect of age on taste sensitivity is controversial. However, the great majority of taste threshold studies suggest a decrease in sensitivity (increased threshold levels) with age-- in both recognition and detection sensory measurements.

In an early report by Richter and Campbell (1940), elderly subjects (52 subjects, aged 52 to 85 years) possessed detection and recognition threshold for sucrose approximately twice as high as the 58 children and 45 young adults. Hinchcliff (1958) also found an age-related deterioration in the detection thresholds for sweet and salty tastes in individuals approaching 70 years of age.

In contrast to the previously cited papers, Byrd and Gertman (1959) failed to find any indication of severe taste impairment in older persons. Using three groups of 20 persons each, (18 to 25, 60 to 70, 80 to 90 years old) the authors compared the subjects' sensitivity to the four basic taste qualities by putting two drops of the tastants on the tongue. Inspection of the data, however, reveals a general decline in the frequency of positive identifications of the four tastes and an increase in the incidence of hypogeusia with age.

Cooper et al. (1959) extended these observations by studying the effect of age on taste sensitivity to the four basic tastes in 100 subjects (15 to 29, 30 to 44, 45 to 59, 60 to 74, 75 to 89 years old). The results clearly showed a decline of taste sensitivity for the four basic tastes after 50 years of age. A later report by Hermel et al. (1970) corroborates these findings that showed decreased sensitivity to sweet, sour and bitter in adults (48 to 60 years) as compared to adolescents (12 to 14 years) and young adults (20 to 25 years).

Balogh and Lelkes (1961) concluded that sensitivity for sweet (sucrose) and salty (NaCl) tastes decreased after age 60, whereas bitter (quinine) tastes became more intense. They also reported confusion of tastes among the elderly, which were attributed to inflammations, wearing of dentures, and leukoplakia.

Kaplan et al. (1965) studied the cumulative effect of age and smoking on taste sensitivity in males and females. No significant age-related differences in taste sensitivity for quinine or for 6-n-propylthiouracil were observed in a sample of 268 non-smoking subjects between the ages of 16 and 55. Taste deterioration was evident, however, in a group of subjects who smoked 20 or more cigarettes per day.

Cohen and Gitman (1959) found that complaints referable to taste perception are far more frequent in the aged (248 institutionalized residents, aged 65 to 94 years), than in the younger, non-institutionalized individuals and bear no relation to the person's ability to recognize the basic tastes. It should be pointed out that the authors tested taste perception by using suprathreshold concentrations of the four basic tastes (saccharine, NaCl, quinine, and acetic acid) which

they painted on the tongue. This somewhat unorthodox method of testing gustation makes an evaluation of their studies somewhat difficult, especially in trying to compare their results with reports of other investigations. Nevertheless, the authors concluded that there is no significant decrement in gross (suprathreshold) taste perception with aging.

A brief report by Schaupp (1970) studied the threshold of the sense of smell and taste in 396 patients who suffered from endocrine diseases; diabetes, thyroid gland diseases - or adrenal gland sicknesses. The author could find no distinct sign of any relationship of taste or olfaction to age in the sensory testings.

Greger (1977) examined dietary intakes, nutritional status, and taste acuity for both detection and recognition thresholds in 65 institutionalized aged subjects. Approximately one-fifth of the subjects had decreased taste acuity as evidenced by an inability to detect a difference in deionized water and 48 mM solutions of sodium chloride or sucrose. In particular, the taste detection threshold for sucrose was greater than 48 mM for 21% of the aged men and 6% of the aged women; and the taste detection for sodium chloride was greater than 48 mM for 17% of the aged men and 18% of the aged women. These values agree closely with a similar study by Greger and Sciscoe (1977) who found that 16% of the 44 elderly (mean age: 69 years), could not detect sodium chloride at 48 mM. The taste thresholds of the elderly subjects were almost twice those observed in three other studies with younger adults.

In yet another study on taste perception in the elderly, Greger and Geissler (1978) studied the effect of zinc supplementation on the

two taste modalities of salty and sweet. Before supplementation, 8% of the aged could not detect a 48 mM solution of sodium chloride, and 6% could not detect a 48 mM solution of sucrose. The detection thresholds for sodium chloride and sucrose improved slightly but not significantly among subjects receiving zinc supplementation. Langan and Yearick (1976), in a study of similar design, tested the effects of improved oral hygiene on taste perception and nutrition of the elderly. Twenty-three subjects, ranging in age from 52 to 86 years, participated in the oral hygiene program conducted over five weeks. Although the subjects varied widely in their ability to detect and identify the four primary tastes, the mean thresholds were generally comparable to those recorded for younger persons. However, the elderly could detect tartaric acid and caffeine at low concentrations, but could not identify the taste as sour or bitter unless the solutions were quite concentrated. Identification thresholds for these two tastes were four to five times as high as those of younger subjects. After the oral hygiene treatment, the 12 elderly demonstrated a significant reduction in their mean detection thresholds for sucrose and sodium chloride.

A recent report on the age-related differences in salt taste acuity of the elderly (Grzegorczyk et al., 1979) criticizes many of the earlier taste threshold studies on the grounds of contradictory methodologies, inappropriate experimental designs, or both. The authors state, "that it is difficult to place confidence in past studies because inadequate psychophysical procedures were used and contradictory results obtained." The researchers comment on the lack of water rinses between tastings which might cause preadaptation to the previous taste solution. Other

researchers used volumes of tastant insufficient to stimulate taste buds in all parts of the tongue and oral cavity (Hinchcliffe, 1959). The blindfolding technique of Cooper et al. (1959) may have caused disorientation for the older individuals. Another criticism of the earlier investigations is the lack of control for response criterion biases, which may result in higher threshold values being reported. In addition, the elderly are known to be more cautious or reluctant to identify the presence of a sensory stimulus; thus, it behooves the experimenter to use a forced-choice, sensory testing procedure.

Grzegorczyk et al.(1979) selected 22 subjects aged 20 to 39 years; 12 subjects, aged 40 to 59 years; 36 subjects, aged 60 to 79 years; and 6 elderly, aged 80 to 92 years. Detection thresholds were measured using the "up-down" or tracking procedure, in which the subject's response determines the next concentration to be tested (Cornsweet, 1962).

The salt taste detection thresholds increased with age, from 23 to 92 years, although the progressive increase in salt threshold was quite small. The large range of thresholds for individuals over 60 years (0.5 to 14.0 mM) demonstrates the large variability among elderly subjects. The authors contend that, with the current state of knowledge concerning the biology of taste receptors, a large increase in threshold as a function of age should not be expected. Indeed, other researchers have noted that age-related differences in taste acuity are small (Murphy, 1979).

Schiffman et al. (1979) examined the detection thresholds for amino acids in two groups of individuals, college students, 17 to 27 years and elderly, 75 to 87 years (mean: 78.4). The elderly subjects possessed taste thresholds for the 19 amino acids 2-1/2 times higher,

on the average, than the young subjects. This decline in sensitivity, Schiffman speculated, is most likely due to decreased numbers of papillae and taste buds per papilla associated with aging.

REFERENCES

1. Balogh, K., and Lelkes, K., The tongue in old age. Gerontol. Clin. 3, 38-54, 1961.

2. Byrd, E., and Gertman, S., Taste sensitivity in aging persons. Geriatrics, 14, 381-384, 1959.

3. Cohen, T., and Gitman, L., Oral complaints and taste perception in the aged. J. Gerontology, 14, 294-298, 1959.

4. Cooper, R.M., Bilash, I., and Zubek, J.P., The effect of age on taste sensitivity. J. Gerontol, 14, 294-298, 1959.

5. Greger, J.L. Dietary intake and nutritional status in regard to zinc of institutionalized aged. J. Gerontology, 32, 549-553, 1977.

6. Greger, J.L., and Sciscoe, B.S., Zinc nutriture of elderly participants in an urban feeding program. J. Am. Diet Assoc., 70, 37-41, 1977.

7. Greger, J.L., and Geissler, M.S., Effect of zinc supplementation on taste acuity of the aged. Am. J. Clin. Nutr., 31, 633-637, 1978.

8. Grzegorczyk, P. B., Jones, S.W., and Mistretta, C.M., Age-related differences in salt taste acuity. J. Gerontology, 34, 834-840, 1979.

9. Hermel, J., Schonwetter, S., and Samueloff, S. Taste sensation and age in man. J. Oral Med., 25, 29-42, 1970.

10. Hincheliff, R. Clinical quantitative gustometry. Acta Otolaryngol., 49, 453-466, 1958.

11. Kaplan, A.R., Glanville, E.V., and Fischer, R., Cumulative effect of age and smoking on taste sensitivity in males and females. J. Gerontology, 20, 334-337, 1965.

12. Langan, M.J., and Yearick, E.S., The effects of improved oral hygiene on taste perception and nutrition of the elderly. J. Gerontology, 31, 413-418, 1976.

13. Murphy, C. The effect of age on taste sensitivity. In: S. Han and D. Coons (Eds.), "Special Senses in Aging: A current biological assessment," Institute of Gerontology, Univ. of Michigan, Ann Arbor, 1979, (in press).

14. Richter, C.P., and Campbell, K.H., Sucrose taste thresholds of rats and humans. Am. J. Physiol., 128, 291-297, 1940.

15. Schaupp, H., Smell and taste in endocrine diseases. Biol. Abstr., 51, 7022, 1970.

16. Schiffman, S.S., Hornack, K., and Reilly, D., Increased taste thresholds of amino acids with age. Am. J. Clin. Nutr., 32, 1622-1627, 1979.

Olfactory Sensitivity

In an early investigation, Chalke and Dewhurst (1957) reported that approximately 30% of the elderly tested (60 persons over 65), could not identify the odor of town gas at concentrations below 50 parts per 10,000. For the younger subjects (under 65), over 95% could smell the gas at 20 parts, and 50% could recognize 8 to 10 parts per 10,000. In a later report, Chalke (1958) found that 30% of persons over 65 cannot recognize town gas by smell. Chalke suggested that the elderly, who possess olfactory abilities may be one-third to one-half less sensitive than those under 65.

Kimbrell and Furchtgott (1963) tested four groups of individuals, (mean ages: 45.9, 54.1, 65.7, 73.9) for olfactory acuity to 20 solutions each of n-butanol and iso-amyl acetate using a forced choice sniffing technique. Thresholds were found to increase with aging for both substances, although the iso-amyl acetate data did not produce a significant F-value, presumably because of a large variability in the oldest age group.

Rovee et al. (1975) studied the olfactory acuity of 120 individuals ranging in age from 6 through 94 years by means of magnitude production in a simple motor task. Seven concentrations of n-propanol, ranging from 1.54% to 100%, served as odorants. Although the authors reported sound evidence of olfactory decline in the oldest participants was lacking, the experimental protocol and conclusions based on their own data suggest the opposite conclusion. The authors

Olfactory Sensitivity

In an early investigation, Chalke and Dewhurst (1957) reported that approximately 30% of the elderly tested (60 persons over 65), could not identify the odor of town gas at concentrations below 50 parts per 10,000. For the younger subjects (under 65), over 95% could smell the gas at 20 parts, and 50% could recognize 8 to 10 parts per 10,000. In a later report, Chalke (1958) found that 30% of persons over 65 cannot recognize town gas by smell. Chalke suggested that the elderly, who possess olfactory abilities may be one-third to one-half less sensitive than those under 65.

Kimbrell and Furchtgott (1963) tested four groups of individuals, (mean ages: 45.9, 54.1, 65.7, 73.9) for olfactory acuity to 20 solutions each of n-butanol and iso-amyl acetate using a forced choice sniffing technique. Thresholds were found to increase with aging for both substances, although the iso-amyl acetate data did not produce a significant F-value, presumably because of a large variability in the oldest age group.

Rovee et al. (1975) studied the olfactory acuity of 120 individuals ranging in age from 6 through 94 years by means of magnitude production in a simple motor task. Seven concentrations of n-propanol, ranging from 1.54% to 100%, served as odorants. Although the authors reported sound evidence of olfactory decline in the oldest participants was lacking, the experimental protocol and conclusions based on their own data suggest the opposite conclusion. The authors

proposed that phylogenetically and ontogenetically early sensitivities (e.g., olfactory, touch) are likely to be among the last to decline when the organism is under stress as, for example, in aging.

In perhaps the most complete study on olfactory thresholds, Venstrom and Amoore (1968) tested 97 individuals for their olfactory response to 18 odorants of known purity. The panel containing 35 persons under 40 years of age achieved a lower threshold than 62 individuals in the older group (over 40 years) for 15 of the 18 odorants. Despite the marked intersubject variation, a general logarithmic decline in sensitivity with age was apparent over the tested range of 20 to 70 years.

The most recent study of olfactory sensitivity in the elderly was detailed by Schiffman (1979). Using an olfactometer that provides six dilution levels, the author found increased thresholds in the elderly subjects for nine commercial food flavors. The 20 elderly subjects (78 to 90 years), demonstrated great difficulty in identifying the various odorants as evidenced by a confusion in classifying five fruit aromas; i.e., cherry, grape, lemon, orange, and tomato.

Further evidence for age-related decrements in olfaction was demonstrated in another experiment by Schiffman (1979). Two groups were selected in an effort to test their sensitivity to eight steroid odors. The young group (17 students aged 18 to 22) performed significantly better at correctly guessing the bottle containing the steroid aroma than the elderly group (aged 72 to 83).

REFERENCES

1. Chalke, H.D., and Dewhurst, J.R., Coal gas poisoning: loss of sense of smell as a possible contributing factor in old people. Br. Med. J., 2, 1915-1917, 1957.

2. Chalke, H.D., Dewhurst, J.R., and Ward, C.W., Loss of sense of smell in old people. Public Health, 72, 223-230, 1958.

3. Kimbrell, G., and Furchtgolt, E., The effect of aging on olfactory threshold. J. Geront., 18, 364-365, 1963.

4. Schiffman, S. Changes in taste and smell with age: Psychophysical Aspects. In: "Sensory systems and communication in the elderly (Aging, Vol. 10)". J.M. Ordy and K. Brizzee, Eds., Raven Press, New York, pp. 227-246, 1979.

5. Rovee, C.K., Cohen, R.N., and Shladack, W., Life-span stability in olfactory sensitivity. Develop. Psy., 11, 311-318, 1975.

6. Venstrom, D., and Amoore, J.E., Olfactory threshold in relation to age, sex or smoking. J. Food Sci., 33, 264-265, 1968.

A.2. Psychological Scaling and Odor Recognition.

A great majority of the sensory evaluation studies in the aged population have concentrated on taste and/or olfactory sensitivity using threshold methodologies. While such a protocol is obviously designed to delineate the underlying physiological taste and olfactory aspects of aging, the more appropriate use of suprathreshold scaling has found little use by gerontologists in the literature. Bartoshuk (1978) has stated that the use of threshold measurements are of limited usefulness because they do not describe the dynamic range of sensory function. In addition, the measurements of threshold can also offer considerable methodological problems to the unwary.

A search of the sensory literature reveals the presence of one article on the influence of age on sweetness perception. In a paper entitled, "Contributions of Age, Sex and Degree of Fatness on Preferences and Magnitude Estimations for Sucrose in Humans", Enns et al. (1979) studied suprathreshold scaling for six levels of sucrose by fifth graders, college students, and elderly persons.

The six sucrose concentrations used in the study (1.95 to 34.23% wt/vol) were dissolved in distilled water and presented to the subjects in plastic medicine cups. Subjects rated the pleasantness of each concentration on a nine point scale which ranged from "extremely pleasant" to "extremely unpleasant". Sweetness intensity was rated by the method of magnitude estimation (ME).

The results indicated that the three classes of subjects exhibited similar mean logs of the ME of sweetness intensity. In fact,

the linear plots of the mean log ME vs. sucrose concentration for the college undergraduates (n=27) and the elderly (n=12) were almost identical.

The mean hedonic ratings of the sucrose concentrations between the elderly and college subjects are more difficult to conceptualize. The female elderly subjects (n=7, mean age: 70.5) demonstrated a decreasing taste function with increasing concentration, while the male elderly subjects (n=5, mean age: 71.60) increased their degree of liking with concentration. The authors concluded that males and females, and not the age groups, differed in their hedonic responses to sucrose solutions, especially within the range of 0.56M-1.0M. In addition, their failure to find a flattened ME function in the elderly strongly suggests that old age does not produce deficits in sensory coding the suprathreshold sweet concentrations.

ODOR RECOGNITION

One of the earliest studies on aroma recognition by the elderly was reported in an article on accidental coal-gas poisoning by Chalke and Dewhurst (1957). The authors, in a preliminary study, asked their subjects (60 persons over 65 years of age) whether they could detect or identify seven common odors (clove, lavender, onion, pear-drops, wintergreen, peppermint, and coal gas). The results indicated that although few subjects were able to identify the odors, a significantly greater proportion were able to detect the odors but not identify them.

In a later report, Anand (1964) studied odor recognition indirectly,

studying the etiology of accidental gas accidents in the homes of elderly subjects living alone. The author surveyed 290 people, of whom 198 were sixty years and older. Four separate test bottles containing coffee, peppermint, coal tar and oil of almonds were used. A fifth bottle, that was empty, served as a check of answer reliability. The authors found a high proportion (89%) of the individuals aged 20 to 39 who were able to identify two or more of the test substances, whereas only 22% of those over the age of 70 were able to do the same. As many as 29% of the people above the age of 80 years could smell nothing at all in any of the test bottles, and another 29% were not able to identify any of the four test substances.

Table 1 summarizes the results of response to odorants tested by Anand (1964). The author speculated that the diminished sense of smell in advancing age is presumably of central origin, due to degenerative changes in the periamygdaloid and pre-piriform areas of the piriform lobe or their association tracts. His view is supported by the present finding that faulty discrimination of smells is more frequent than complete absence of smell.

Schiffman (1977) updated the research in food recognition by the elderly in a recent study of 27 college students and 29 elderly subjects who tasted and smelled a series of unseasoned, blended foods. As in the earlier studies cited above, the elderly subjects were significantly less able to identify the foods than the younger subjects. Table 2 summarizes the more important food substances and the percentage of subjects correctly identifying them.

The results of Schiffman's (1977) experiments suggest a considerable loss of accuracy of food recognition in the elderly based on cues

Table 1. Response of 290 subjects to four common odors. Adapted from Anand (1964)

Age (years)	Number of Subjects	% Who Smelled Nothing	Percentage of Subjects in each age-group who identified the substances correctly[1]				
			0	1	2	3	4
20-39	52	0	8	4	8	46	35
40-59	40	0	15	0	30	35	20
60-69	48	8	27	27	27	6	4
70-79	87	10	31	34	15	6	3
Over 80	63	29	29	24	19	0	0

[1] 0=empty bottle, 1=coffee, 2=peppermint, 3=coal tar, 4=oil of almonds

Table 2. Percentage of subjects correctly identifying each of several selected blended foods. Adapted from Schiffman (1977).

Food Substance	Elderly Citizens (%)	College Students (%)
Lemon	24	52
Strawberry	33	78
Carrot	7	63
Cabbage	7	4
Potato	38	19
Tomato	69	52
Fish	59	78
Coffee	70	89
Salt	89	89
Sugar	57	63

of taste and smell. The decline in olfactory sensitivity is relatively more noticeable than the decline in taste sensitivity. The researchers noticed that significantly more elderly subjects commented on the weakness of lack of taste or smell of the foods than the younger subjects.

In a later report on the decreased discrimination of food odors in the elderly, Schiffman and Pasternak (1979) reiterated their findings of the lessened ability of the elderly to differentiate between simulated food odors varying in aroma type.

The 16 elderly subjects, aged 72-78 years, were best at discriminating fruits (lime, strawberry, apple, grape, cherry, tomato, etc.) from the rest of the stimuli (bacon, chicken, beef, butter, chocolate). In contrast to Schiffman's earlier study, the subjects merely sniffed the commercial food odorants from 4-oz wide-mouthed bottles. The authors concluded that since many of the elderly no longer have the ability to accurately discriminate food odors, they need to place more emphasis on texture and visual cues for the correct identification of foods.

Schiffman (1979) described two other studies involved with the detection of food and steroid odors in the elderly. In the first study, Schiffman et al.(1976) found increased thresholds in elderly subjects for nine commercial food flavors using an olfactometer that controls for guessing. The thresholds for the 20 elderly subjects (78 to 90 years) and young controls (20 to 26 years) demonstrated a great difference in olfactory acuity.

In the second study, Schiffman et al. (1977) compared the ability to detect eight steroid odors between 17 college students (18 to 22

years) and 23 elderly (72 to 83 years). Subjects were required, while blindfolded, to say which bottle had a smell. For the 23 aged subjects, the average number of correct responses was very small compared to the college students. In another experiment, Schiffman studied the ability of young and old subjects to detect a musk odor. The 13 elderly subjects not only possessed decreased sensitivity to musk odor, but also 44% of the responses for the elderly were false-positives compared to 16% for the youthful subjects.

The obvious decline in olfactory sensitivity in the aged suggested a follow-up study by Schiffman (1979), where hedonic ratings of food were studied after being amplified by the addition of imitation flavors. Five blended foods were fortified with artificial flavor to "moderately strong" and "very strong" levels. Fourteen elderly subjects (77 to 84 years) and 11 college students (17 to 25 years) tasted and smelled blended foods and rated them along a 5-inch line labeled "good" at one end and "bad" at the other. The results show that amplification of the flavor above the natural blended forms increased hedonic ratings for the elderly, but not the college subjects. The elderly subjects, in general, made very favorable comments about the flavor of amplified foods, whereas the younger subjects made frequent unfavorable comments relating to overpowering odors and unpleasant tastes.

REFERENCES

1. Anand, M.P., Accidents in the home. In: "Current Achievements in Geriatrics"; E.D.S., Anderson, W.F., and Isaacs, B. pp. 239-245, 1964.

2. Bartoshuk, L.M., The psychophysics of taste. Am. J. Clin. Nutr., 31, 1068-1077, 1978.

3. Chalke, H.D. and Dewhurst, J.R., Coal gas poisoning: Loss of sense of smell as a possible contributory factor with old people. Br. Med. J., 2, 1915-1917, 1957.

4. Enns, M.P., Van Itallie, T.B. and Grinker, J.A., Contributions of age, sex and degree of fatness on preference and magnitude estimations for sucrose in humans. Physiol. Behav., 22, 999-1003, 1979.

5. Schiffman, S.S., Moss, J. and Erickson, R.P., Thresholds of food odors in the elderly. Exp. Aging Res., 2, 389-398, 1976.

6. Schiffman, S., Food recognition by the elderly. J. Gerontol., 32, 586-592, 1977.

7. Schiffman, S., Changes in taste and smell with age: Psychophysical aspects. In: "Sensory Systems and Communication in the Elderly", Aging, Vol. 10. EDS., Ordy, J.M. and Brizzee, pp. 227-246, K. Raven Press, New York, 1979.

8. Schiffman, S. and Pasternak, M., Decreased discrimination of food odors in the elderly. J. Gerontol.,34, 73-79, 1979.

9. Schiffman, S., Stauros, P. and Pasternak, M., Change in sensitivity to urinoid and musk odors with age. Unpublished manuscript.

A.3. Salivary Flow and Composition

Few reports documenting the effects of aging on salivary flow rates are available. In a comprehensive review of human salivation and psychophysiology, Brown (1970) states that unstimulated salivation is profuse in infancy, declines rapidly up to age 5, and then falls off more gradually until puberty.

One early report by Meyer et al., (1937) studied the ptyalin content of human saliva in a group of 12 subjects with an average age of 25 years and a group of 27 subjects with an average age of 82 years. The researchers noticed that some of the elderly were not able to chew and expectorate saliva for the entire period of 30 minutes because of dryness of mouth. The volume of stimulated saliva collected for 30 minutes in the younger group varied between 13.6 and 15 cc with an average of 14.2 cc, while that of the older group varied between 1.5 and 15 cc with an average of 5.8 cc. Finally, the older group averaged 0.33 units of ptyalin per cc of saliva compared with 10.15 units for the younger group.

The most comprehensive study of salivary flow in healthy individuals was conducted by Beck and Wainwright (1943). The research protocol used 661 individuals, aged 5 to 95 years, who submitted resting salivary samples in the morning prior to brushing. The resting salivary data revealed a wide scattering of values throughout all ages with a decreased incidence of fast rates of flow in the older age groups. The highest rate of flow included the 25 to 29 year old group and the lowest in the 5 to 9 group.

In another early study, Wainwright (1943) reported on the inorganic phosphorus content of resting saliva in 650 individuals aged 5 to 95 years. The author discovered a gradual rise in the phosphorus mg % values with increasing age. Furthermore, the mean rate of flow of 661 individuals was 19 ± 0.54 cc/hr. with little correlation between age and flow rate. No sex differences were found between the average flow rates of males, 20 ± 0.81 cc/hr. and females, 19 ± 0.96 cc/hr. There was, however, less of a range in the rate of flow values for individuals 50 to 95 as compared to the 5 to 49 year old group.

More recently, Gutman and Ben-Aryeh (1974) studied the influence of age (from 6 to 76 years) on salivary rate of flow and electrolyte content (Na, K, Ca, and Mg). The mean rate of flow for the older age group (60-76 years) was the lowest (0.37 ml/min.) while the children and young adults averaged 0.65 and 0.41 ml/min., respectively. The mean salivary content of sodium, potassium and magnesium was higher in the older age group. The authors suggested that salivary gland function decreased with age which may imply a change in the activity of salivary enzymes. The higher concentration of electrolytes found in aging individuals by Gutman and Ben-Aryeh (1974) were confirmed earlier by Grad (1954), who found that aged men 40 to 90 years had a significantly higher Na concentration in saliva than men under 40. However, the salivary Na content of women 40 years or older was not statistically different from that of women under 40.

Mäkilä (1977), in a study of the salivary secretions of elderly institutionalized patients, concluded that degenerative changes in salivary glands, multiple illnesses, and recurrent use of medication are reasons for a lower rate of salivary secretion among the elderly.

Among the 105 men tested, the mean secretion rate was 1.20 ml/min., as compared to a mean of 0.98 ml/min. for the 295 women. As the number of diseases experienced by the patients increased (0 to 6 diseases), the rate of salivary secretion diminished by up to 15 percent. Subjects who wore dentures exhibited a faster flow rate than those who did not. While viscosity values did not change with increasing age, they did increase in individuals with atrophic changes in the mucous membranes.

Several researchers have attempted to delineate the etiology of reduced salivary flows using histochemical or morphological techniques (Scott, 1975 and 1977b; Courtney, 1972; and Andrew, 1952). Andrew (1952) compared the age changes in 31 human parotid and submandibular salivary glands procured from surgical specimens. Destructive fatty replacement seen in old age occurs in the parotid gland, but less so in the submandibular gland. Accumulations of lymphoid tissue are common in the salivary glands in old age in man as well as in the rat.

Scott (1975) undertook an investigation to define the normal range of adult human submandibular gland volumes and to determine the extent of age, sex and contralateral variability. The volumes of 153 glands were taken from 70 necropsied subjects between the ages of 16-95 years. The results indicated wide variation found both between individuals and between sides in the same individual. Male submandibular glands were larger than female glands by at least 50 percent. In old age, where general atrophy of many organs is present, there was some reduction in gland volume.

Scott (1977b) extended these observations to 82 submandibular glands that were examined histologically for age changes in the ducts. While the striated duct system remained a constant 5 percent of the

total gland volume with increasing age, the absolute proportion of striated ducts decreased. The duct system occupied by both the non-striated intralobular ducts and by the extralobular ducts increased with advancing age. This presence of a larger duct system with age may reduce salivary flow rate as it results in prolonged contact time between saliva and duct epithelium during secretion, causing increased osmotic reabsorption of water. The increased numbers of degenerative or involuted forms of duct epithelium observed in aging might also contribute to decreased salivary flow.

Courtney (1972) examined the effect of age on the periodontium and oral mucous membranes of elderly subjects. A classic symptomatology of aging, i.e., oral mucosa which is dry, thin, and easily abraded, is associated with reduced or absent salivary flow. Observed changes in the salivary gland with age, including fatty atrophy, fibrosis, reduced parotid parenchyma and increasing numbers of unusual cells called oncocytes, may be responsible. Scott (1977b) also noted a reduction of the volume of parenchyma with increasing age, while, at the same time, the proportions of other submandibular tissues increased. This loss of acinar tissue--with an increase of duct tissue--greatly increased in the final decades of life (80-90 years).

REFERENCES

1. Andrew, W. A Comparison of Age Changes in Salivary Glands of Man and of the Rat. J. Gerontol. 7, 178-190, 1952.

2. Bauer, W.H. Old Age Changes in Human Parotid Glands with Special Reference to Peculiar Cells in Uncommon Salivary Gland Tumors. J. Dental Research, 29, 686-687, 1950.

3. Becks, H, and Wainwright, W.W. Human Saliva: XIII. Rate of Flow of Resting Saliva of Healthy Individuals. J. Dental Research 22, 391-396, 1943.

4. Brown, C. The Parotid Puzzle: A Review of the Literature on Human Salivation and its Applications to Psychophysiology. Psychophysiology 7, 66-85, 1970.

5. Courtney, R.M. Age Changes in the Periodontium and Oral Mucous Membranes. J. Michigan Dental Assoc. 54, 335-344.

6. Grad, B. Diurnal, Age and Sex Changes in the Sodium and Potassium Concentrations of Human Saliva. J. Gerontol. 9, 276-86, 1954.

7. Gutman, D., and Ben-Aryeh, H. The Influence of Age on Salivary Content and Rate of Flow. Int. J. Oral Surgery 3, 314-317, 1974.

8. Mäkilä, E. Oral Health among the Inmates of Old People's Homes. Salivary Secretion. Proc. Finn. Dent. Soc. 73, 64-69, 1977.

9. Meyer, J., Golden, J.S., Steiner, N., and Necheles, H. The Ptyalin Content of Human Saliva in Old Age. The Am. J. of Physiology 119, 600-602, 1937.

10. Scott, J. Age, Sex and Contralateral Differences in the Volumes of Human Submandibular Salivary Glands. Arch. Oral Biology 20, 885-887, 1975.

11. Scott, J. A Morphometric Study of Age Changes in the Histology of the Ducts of Human Submandibular Salivary Gland. Arch. Oral Biology 22, 243-249, 1977a.

12. Scott, J. Quantitative Age Changes in the Histological Structure of Human Submandibular Salivary Glands. Arch. Oral Biology 22, 221-227, 1977b.

13. Wainwright, W.W. Human Saliva XV. Inorganic Phosphorus Content of Resting Saliva of 650 Healthy Individuals. J. Dental Research 22, 403-414, 1943.

B.1. Dietary Recalls, Food Intake and Nutritional Surveys of Elderly and College-Aged Students

a. Elderly Subjects

During the last decade, the general population of the United States increased in the percentage of individuals aged 30 years and older (Fowles, 1975). Accurate information concerning the dietary practices and habits of this maturing population becomes of utmost importance in the determination and implementation of nutritional care practices everywhere.

In a recent editorial, Hamish Munro (1980) addresses these concerns when he states that we have little knowledge of optimal nutrient intakes for those who are already old. Munro contends that a major phenomenon of aging is the progressive reduction in voluntary intake of energy due to loss of active tissue resulting from reduced physical activity. This reduction in the needs for energy decreases total food intake which presumably affects most nutrients.

Several investigators in the U.S. and elsewhere have surveyed the energy intakes of the elderly. For example, in one study by McGandy *et al.* (1966) was aimed at businessmen and executives over the range of 20 to 99 years of age. The results indicate that energy intake over the age of 65 averages 2,300 kcal per day. In contrast, a study by Dibble *et al.* (1967) of inner city men in Syracuse showed a much lower average energy intake than the Ten-State Nutrition Survey did for elderly men (DHEW Pub., 1972).

A consideration of a few critical nutrients illustrates the

importance of establishing allowances of guidelines for the elderly population. The allowance of 45 mg Vit. C was recommended for adults of all ages in the 9th revision of the Recommended Dietary Allowances (RDA's) (Food and Nutrition Board, 1980). In all but two of the 14 studies where Vit. C intake was cited in Table 3, the elderly met or exceeded this recommendation. Even the early studies of Vit. C intake (Dibble et al., 1967; Bovit, 1965) which originally reported that Vit. C levels were inadequate in the elderly, are adequate based on the new allowance. Only Kelley et al. (1957) and Henrickson and Cate (1971) reported borderline intakes of Vit. C in their subjects.

The consensus of the literature indicates that iron deficiency is more of a problem in elderly women than in elderly men. With an established RDA of 10 mg for men and postmenopausal women, four studies demonstrated marginal iron intakes in their elderly populations (Dibble et al. 1967, Bovit, 1965; Guthrie et al., 1972; Henrickson and Cate, 1971).

Adequate levels of calcium and Vitamin D intake in the elderly are necessary to prevent the occurrence of osteoporosis and fractures of the vertebrae and neck. An RDA of 800 mg of calcium was not an easy goal to attain for many elderly women. Seven studies reported lower intakes of this nutrient than the minimum RDA (Joering, 1971; Grotkowski and Sims, 1978; Dibble et al., 1967; Kohrs et al., 1978).

Although there is dispute regarding the protein allowances for adults (Munro, 1980), most of the cited studies found that the elderly easily met or exceeded the low 44 g RDA. However, because these adults tend to consume fewer kilocalories than their younger counterparts, the intakes of individual nutrients, especially the mineral elements, will continue to be of concern. Table 3 summarizes the results reported by

various investigators on nutrient intakes in the elderly based on dietary recalls.

Table 3. Comparison of Reported Intakes of Selected Nutrients with the Recommended Daily Dietary Allowances (RDA)

Nutrients	1980 RDA (men)	1980 RDA (women)	AUTHORS: Abdulla et al. (1977) (men)	Abdulla et al. (1977) (women)	Bovit (1975) % 1963 RDA
Kilocalories	2050	1600			81
Protein	56	44 g			81
Fat					
Carb.					
Cholesterol					
Fiber					
Vit. C	60	60 mg			71
Thiamin	1.2	1.0 mg			83
Niacin	16	13.0 mg			
Riboflavin	1.4	1.2 mg			89
Pyridoxine	2.2	2.0 mg			
B_{12}	3.0	3.0 mcg			
Folic Acid	400	400 mcg			
Vit. A	10000	8000 IU			81
Vit. D	5	5 mcg			
Iron	10	10 mg	13.3	9.8 mg	81
Calcium	800	800 mg	725.0	610.0 mg	68
Phosphorus	800	800 mg			
Sodium			88.0	77.0 mg	
Potassium			53.0	44.0 mg	
Magnesium	350	300 mg	210.0	170.0 mg	
Copper	2.0	2.0 mg	1.25	0.97 mg	
Zinc	15.0	15.0 mg	8.25	7.2 mg	
Sugars					

Table 3 (continued)

Nutrients	AUTHORS: Deeming and Weber (1978) Men	Deeming and Weber (1978) Women	Dibble et al. (1967) Men	Dibble et al. (1967) Women	
Kilocalories			1643	1467	
Protein			63.3	57.5	g
Fat			67.0	54.0	g
Carb.			214	168	g
Cholesterol					
Fiber					
Vit. C			50	54	mg
Thiamin			0.98	0.88	mg
Niacin					
Riboflavin			1.33	1.31	mg
Pyridoxine					
B_{12}					
Folic Acid					
Vit. A			5348	5719	IU
Vit. D					
Iron	22 mg (men + women)		10.3	8.7	mg
Calcium			0.89	0.64	g
Phosphorus					
Sodium					
Potassium					
Magnesium	262 mg (men + women)				
Copper					
Zinc	15	1.7 (M+W) 8 mg			
Sugars					

Table 3 (continued)

Nutrients	AUTHORS: Greger and Geissler (1978)	Grotkowski and Sims (1978) Men	Grotkowski and Sims (1978) Women
Kilocalories	2377	1801	1363
Protein	105 g	80.4	568 g
Fat		80.0	54.7 g
Carb.		195.4	167.6 g
Cholesterol			
Fiber			
Vit. C	128 mg	110.0	76.3 mg
Thiamin	1.5 mg	1.34	0.96 mg
Niacin	17.0 mg	16.2	11.92 mg
Riboflavin	2.9 mg	1.78	1.43 mg
Pyridoxine			
B_{12}			
Folic Acid			
Vit. A	11485 IU	5104	4712 IU
Vit. D			
Iron	14.9 mg	14.5	10.5 mg
Calcium	1517 mg	799	536 mg
Phosphorus			
Sodium			
Potassium			
Magnesium			
Copper			
Zinc	12.3 mg		
Sugars			

Table 3 (continued)

Nutrients	AUTHORS					
	Guthrie et al. (1972)			Henrickson and Cate (1971)		
	Men	Women		Men	Women	
Kilocalories	1681	1347		1475	1258	
Protein	67	56	g	59	51	g
Fat						
Carb.						
Cholesterol						
Fiber						
Vit. C	50.7	63.5	mg	45	43	mg
Thiamin	1.0	0.9	mg	0.8	0.8	mg
Niacin						
Riboflavin	1.3	1.3	mg	1.2	1.1	mg
Pyridoxine						
B_{12}						
Folic Acid						
Vit. A	2999	2999	IU	8081	5246	IU
Vit. D						
Iron	11.6	9.6	mg	11.0	9.0	mg
Calcium	568	493	mg	0.6	0.5	mg
Phosphorus						
Sodium						
Potassium						
Magnesium						
Copper						
Zinc						
Sugars						

Table 3 (continued)

Nutrients	AUTHORS					
	Joering (1971)			Justice et al. (1974)		
	Men	Women		Men	Women	
Kilocalories	1921	1388		1609	1388	
Protein	86	69	g	64.8	55.6	g
Fat				65.1	56.4	g
Carb.				200	171	g
Cholesterol						
Fiber						
Vit. C	62.0	55.0	mg	127.0	97	mg
Thiamin	0.90	1.80	mg	1.1	0.9	mg
Niacin	16.0	14.0	mg	11.2	9.5	mg
Riboflavin	1.85	1.40	mg	1.6	1.4	mg
Pyridoxine						
B_{12}						
Folic Acid						
Vit. A	6658	11232	IU	7493	6866	IU
Vit. D						
Iron	13.5	14.1	mg	10.6	9.2	mg
Calcium	935	614	mg	769	684	mg
Phosphorus						
Sodium						
Potassium						
Magnesium						
Copper						
Zinc						
Sugars						

Table 3 (continued)

Nutrients	AUTHORS: Kelly et al. (1957)	Kohrs et al. (1978) Men	Kohrs et al. (1978) Women	
Kilocalories		2342	1619	
Protein	Intakes of	88	62	g
Fat	less than	106	75.2	g
Carb.	40% RDA:	261	177	g
Cholesterol	Calcium,			
Fiber	Vit. A,			
Vit. C	Vit. C	135	124	mg
Thiamin		1.4	1.0	mg
Niacin		19.7	12.0	mg
Riboflavin		2.3	1.7	mg
Pyridoxine				
B_{12}				
Folic Acid				
Vit. A		11645	10648	IU
Vit. D				
Iron		15.0	10.5	mg
Calcium		1095	811	mg
Phosphorus				
Sodium				
Potassium				
Magnesium				
Copper				
Zinc				
Sugars				

Table 3 (continued)

Nutrients	AUTHORS: Kohrs et al. (1978b) MEAN % RDA	AUTHORS: Langan and Yearick (1976)	
Kilocalories	85.1	1378	
Protein	137.2	57	g
Fat			
Carb.			
Cholesterol			
Fiber			
Vit. C	167.5	78	mg
Thiamin	101.7	0.85	mg
Niacin	105.2	11.2	mg
Riboflavin	130.0	1.11	mg
Pyridoxine			
B_{12}			
Folic Acid		121	mcg
Vit. A	118.9	7410	IU
Vit. D	112.7		
Iron		10.4	mg
Calcium	98.6	456	mg
Phosphorus			
Sodium			
Potassium			
Magnesium			
Copper			
Zinc			
Sugars			

Table 3 (continued)

Nutrients	AUTHORS		
	Marshall et al. (1975)		McGandy and Weber (1976)
	Men	Women	Men
Kilocalories	2400	1830	2093
Protein	100.4	75.3 g	81 g
Fat	100.6 g		86 g
Carb.	314.9 g		244 g
Cholesterol	236 mg		480 g
Fiber	7.4 g		
Vit. C	167	126 mg	119 mg
Thiamin	1.93	1.45 mg	1.20mg
Niacin	29.1	21.9 mg	15.0 mg
Riboflavin	1.83	1.37 mg	1.87mg
Pyridoxine	3.0	2.2 mg	
B_{12}	6.8	5.1 mcg	
Folic Acid	221.0	116.0 mcg	
Vit. A	11495	8623 IU	8100 IU
Vit. D			
Iron			12.3 mg
Calcium	670	502 mg	
Phosphorus	1225	942 mg	
Sodium			
Potassium			
Magnesium	256	192 mg	
Copper	0.92	0.69 mg	
Zinc	17.2	12.9 mg	
Sugars	10.1	7.5 mg	

Table 3 (continued)

Nutrients	AUTHORS: Steadman et al. (1980) Men	Steadman et al. (1980) Women	Tucker et al. (1958)
Kilocalories	1720	1333	2283
Protein	67.3	48.0 g	69 g
Fat	67.0	54.0 g	
Carb.	214	168 g	
Cholesterol			
Fiber			
Vit. C	65	83 mg	70.9 mg
Thiamin	1.04	0.67 mg	1.51 mg
Niacin	12.7	7.6 mg	10.5 mg
Riboflavin	1.68	1.24 mg	1.78 mg
Pyridoxine			
B_{12}			
Folic Acid			
Vit. A	5500	4500 IU	7075 IU
Vit. D			
Iron	12.3	7.8 mg	14.47 mg
Calcium	0.89	0.64 g	0.951mg
Phosphorus	1.22	0.87 g	
Sodium			
Potassium			
Magnesium			
Copper			
Zinc			
Sugars			

REFERENCES

1. Abdulla, M., Jagerstad, M., Nordlin, A., Quist, I. and Svensson, S., Dietary Intake of electolytes and trace elements in the elderly, Nutr. Metab., 21, 41-44, 1977.

2. Bovitt, C.D., The food of older persons living at home, J. Am. Diet. Assoc., 46, 285-289, 1965.

3. Dibble, M.V., Brin, M., Thiele, U.F., Peel, A., Chen, N. and McMullen, E., Evaluation of the nutritional status of elderly subjects with a comparison between fall and spring., 15, 1031-1060, 1967.

4. Food and Nutrition Board, National Research Council. "Recommended Dietary Allowances", Nineth revised edition. Washington, D.C. National Academy of Sciences, 1980.

5. Fowles, D.G., U.S. 60^{+} population may rise 31% to 41 million by year 2000. Aging, 248, 14-15, 1975.

6. Greger, J.L. and Geissler, A.H., Effect of zinc supplementation on taste acuity of the aged. J. Am. Clin. Nutr., 31, 633-637, 1978.

7. Grotkowski, M.J. and Sim, L.S., Nutritional knowledge attributes, and dietary practices of the elderly. J. Am. Diet. Assoc., 72, 499-506, 1978.

8. Guthrie, H.A., Black, K. and Madden, P.J., Nutritional practices of elderly citizens in rural Pennsylvania, Gerontol., 12, 3300-3305, 1972.

9. Henricksen, B. and Cate, H.D., Nutrient content of food served vs. food eaten in nursing homes. J. Am. Diet. Assoc., 59, 126-129, 1971.

10. Joering, E., Nutrient contribution of a meals program for senior citizens. J. Am. Diet. Assoc., 59, 129-132, 1971.

11. Justice, C.L., Howe, J.M. and Clark, H.E., Dietary intakes and nutritional status of elderly patients. J. Am. Diet. Assoc., 639-646, 1974.

12. Kelley, L., Ohlson, M.A. and Harper, L.J., Food selection and well-being of aging women. J. Am. Diet. Assoc., 33, 466-470, 1957.

13. Kohrs, M.B., O'Neal, R., Preston, A., Eklund, D. and Abrahams, O., Nutritional status of elderly residents in Missouri. J. Am. Clin. Nutr., 31, 2186-2197, 1978a.

14. Kohrs, M.B., O'Hanlon, P. and Eklund, D., Title VII-Nutrition program for the elderly. J. Am. Diet. Assoc., 72, 487-493, 1978b.

15. Kohrs, M.B., O'Hanlon, P., Krause, G. and Nordstrom, J., Title VII-Nutrition program for the elderly. J. Am. Diet. Assoc., 75, 537-542, 1979.

16. Marshall, M.W., Iacono, J.M., Young, C.W., Washington, V.A., Slover, H.T. and Leapley, P.M. Composition of diets containing 25 and 35 percent calories from fat. J. Am. Diet. Assoc., 66, 470-481, 1975.

17. McGandy, R.B., Barrows, C.H., Spanias, A., Meredith, A. Stone, J.L. and Norris, A.H., Nutrient intakes and energy expenditure in men of different ages. J. Gerontol., 21, 581-587, 1966.

18. Munro, H., Major gaps in nutrient allowances. J. Am. Diet. Assoc., 76, 137-141, 1980.

19. Sandstead, H., Levay, L., Munuz, J., Jacob, R., Logan, G., Dintzis, F., Inglett, G. and Shvey, W. Human zinc requirements: effects of dietary fiber on zinc metabolism. Fed. Proc., 37, 254, 1978.

20. Stiedman, M., Jansen, C. and Harrill, I., Nutritional status of elderly men and women. J. Am. Diet. Assoc., 73, 132-139, 1978.

21. Taper, L.J., Burke, D.M., DeMicco, F.J. and Richey, S.J., Zinc and copper interaction and retention in elderly human subjects. Fed. Proc., 39, 430, 1980.

22. Tucker, R.E., Brine, C.L. and Wallace, M.S. Nutritive intake of older institutionalized persons. J. Am. Diet. Assoc., 34, 819-822, 1958.

23. Wolf, W. R., Holden, J. and Greene, F.E., Daily intake of zinc and copper from self selected diets. Fed. Proc., 36, 1175, 1977.

B.2. Nutritional Status of Zinc/Copper in the Elderly and College-Aged Subjects

Elderly Subjects

Recent innovations in atomic absorption instrumentation (AAS) have allowed the investigator to accurately determine zinc and copper levels in biological systems and foodstuffs. Reliable quantitative values for the zinc and copper constituents of food were first provided by the provisional tables of Freeland and Cousins (1976) and Murphy et al. (1975). Since that time, several studies have focused on the zinc and copper contents of the diets of elderly Americans.

One of the earliest papers in the literature on the zinc nutriture of the elderly is the report by Greger and Sciscoe (1977). Prior to the time of their report, little or no data had been collected on dietary zinc intakes in relation to the nutritional status of the elderly. The authors find this alarming, for they hypothesize that marginal consumption of mineral constituents over a span of sixty years may account for some of the degenerative changes associated with aging.

The forty-four elderly participants in a nutritional survey by Greger and Sciscoe (1977) were classified as being retired, usually living alone, or often reporting some disease condition, particularly arthritis, hypertension, and/or heart disease. The authors found that on an average weekday, the Recommended Dietary Allowance (RDA) for all nutrients, except zinc, was met. Fifty-nine percent of the subjects

consumed less than two-thirds of the allowance for zinc. Low hair zinc levels in eight percent and decreased taste acuity for saltiness in sixteen percent of the subjects suggested that the zinc nutriture may be less than ideal. However, on the basis of hair zinc levels ($\bar{X}$= 174±13 mcg/g) and total average zinc intake (10.1 mg/day), the elderly participants cannot be considered zinc deficient.

In a later report, Greger surveyed the dietary intakes in regard to zinc in 65 members of a state-run institution for the aged. Dietary intakes, compared to the RDA, were generally adequate for all nutrients, except for zinc and magnesium. Five percent of the subjects had hair zinc levels indicative of zinc deficiency (below 75 mcg/g) and one-fifth of the subjects had decreased taste acuity as evidenced by an inability to detect a difference in deionized water and 48 mM solutions of salt or sucrose.

Greger and Geissler (1978), in a study analyzing the effect of zinc supplementation on taste acuity of the aged, calculated the nutrient contents for each day of a 95-day rotating menu which was served to the elderly. The authors calculated the nutrient content of the menu using the United States Department of Agriculture food composition tables, with the aid of the zinc tables by Murphy et al. (1975) and Freeland and Cousins (1976). The diet served by the institution during the supplementation period met or exceeded the RDA for all nutrients measured, with the exception of zinc.

Only two reports have attempted to analyze the copper contents of the diet in conjunction with the zinc values. The dietary intake of electrolytes and trace elements in the elderly was studied by Abdulla et al. (1977). Daily food samples were collected from 17 males and 20 females (67 year old pensioners) using the duplicate portion

technique of Borgstrom et al. (1955). Abdulla et al. found that, except for calcium, the dietary intake of the sodium, potassium, magnesium, iron, zinc and copper was low as compared to the recommended daily dietary allowances. The men in the study averaged 8.25 mg/day zinc and 1.25 mg/day copper, while the females averaged 12.0 mg/day zinc and 1.97 mg/day copper.

The levels of zinc and copper in the self-selected diets of twenty-two men and women were reported by Holden et al. (1979). By using AAS on homogenized wet-ashed food samples, the authors discovered that 68 percent of the subjects consumed less than two-thirds of the recommended daily allowance for zinc (15 mg). Eighty-one percent consumed less than two-thirds of the suggested level of copper (2.0 mg/day). The author concluded that diets that supply adequate amounts of energy and protein do not guarantee adequate levels of zinc and copper.

REFERENCES

1. Abdulla, M., Jagerstad, M., Norden, A., Quist, I. and Svensson, S., Dietary Intake of Electrolytes and Trace Elements in the Elderly. Nutr. Metab., 21, (Suppl. 1): 41-44, 1977.

2. Freeland, J.H. and Counsins, R.J., Zinc content of selected foods. J. Am. Diet. Assoc., 68, 526-529, 1976.

3. Greger, J.L. Dietary Intake and Nutritional Status in Regard to Zinc of Institutionalized Aged. J. Gerontol., 32, 549-553, 1977.

4. Greger, J.L. and Sciscoe, B.S. Zinc nutriture of elderly participants in an urban feeding program. J. Am. Diet. Assoc., 70, 37-41, 1977.

5. Greger, J.L. and Geissler, A.H. Effect of zinc supplementation on taste acuity of the aged. J. Am. Clin. Nutr., 31, 633-637, 1978.

6. Holden, J.M., Wolf, W.R. and Mertz, W. Zinc and copper in self-selected diets. J. Am. Diet. Assoc., 75, 23-28, 1979.

7. Murphy, E.W., Willis, B.W. and Watt, B.K. Provisional tables on zinc content of foods. J. Am. Diet. Assoc., 66, 345-355, 1975.

College Students

Few studies in the literature expressly detail the nutritional contents of diets eaten by college students. It is the age of the college group, not the collegiate status, that concerns most investigators in their selection of a suitable population. For the purposes of this review, nutrient intakes of people between 20 to 30 years (college age) were used.

The earliest report by Tribble and Scoular (1954) studied 13 college women, 17 to 27 years of age, while living in a dormitory-like duplex. The self-selected diets furnished from 9.8 to 14.4 mg of zinc, the average being 13 mg, based on dietary recall and calculations of nutrients in foods eaten.

A later report by White (1969) analyzed 24-hr. weighed diet composites in 21 college women who averaged 20 years of age. The average daily dietary levels of copper, zinc and manganese were 0.50, 13.23 and approximately 1.0 mg, respectively. Only two subjects attained the 2.0 mg copper level suggested by the Food and Nutrition Board in 1980. With the zinc diets, the average values ranged from 1 to 20 mg, with one diet reaching 47.6 mg, presumably from oyster inclusion in the diet.

White (1976) analyzed the mean zinc contents of weighed diets of 15 high school girls and of 30 college women. The range of values for the self-selected diets was quite large, from 4.8 to 47.0 mg/day for the college women, while the mean energy value of the women's diets was low (1467 kcals) and the mean zinc value was intermediate at 13.8 mg. Eleven college women consumed more than 15 mg of zinc per day, 15 women from 10 to 15 mg, and 7 women had diets less than 10 mg per day.

The authors noted that energy restriction and choice of foods significantly influenced zinc intakes.

Walker and Page (1977) sampled meals from fifty colleges in 31 states. Colleges were included only if they provided food service seven days a week. The sample from each college consisted of four breakfasts, four noon meals, and four evening meals for seven consecutive days. The daily zinc content of the college meals ranged from 6.36 to 16.11 mg, with the average being 11.03 mg. The copper content of the meals averaged 3.37 mg (with a range of 1.74 to 12.51 mg). The authors concluded that the meals for the 19 to 21 year old men and women were marginal in zinc levels but quite adequate in copper.

A dietary study by Lyons et al. (1979) of two college students who were residents of western Scotland reported that the 23 year old male consumed 10.1 mg of zinc daily and the 23 year old female 7.6 mg of zinc. The author suggested that diet choices should be made to elevate the trace mineral levels of the diets. Table 4 summarizes the results of the zinc/copper dietary surveys found in the literature.

Table 4. Studies of Zinc/Copper and dietary intake in college students.[1]

Authors	Subjects	Method	Zn (mg)	Cu (mg)
Harland and Peterson (1978)	16 males, ages 26-86, $\bar{X}$: 46.5	Chemical Analysis (CA)	(5-18)	(2.2-2.8)
Harland et al., (1980)	representative two-week analysis of a 15 to 20 yr. old male.	CA	$\bar{X}$: 18.7	(Assumes 3402 kcal intake)
Holden et al., (1979)	22 subjects, 14-64 years	CA	(2.5-19.4) $\bar{X}$:8.16	(0.24-2.52) $\bar{X}$:1.0
Hunt et al., (1979)	344 women $\bar{X}$:25.5 (14-42 years)	Dietary Recall (DR)	$\bar{X}$:9.4 (1-25)	
Lyon et al., (1979)	1 male and female (23 years)	CA	$\bar{X}$:10.1 (male) $\bar{X}$: 7.6 (female)	
Milne et al., (1980)	5 men, 19 to 27 yrs. old	CA	(7.2-42.9) $\bar{X}$:20.3	(0.6-8.5) $\bar{X}$:1.5
Tibble and Scoular (1954)	13 women, 17 to 27 years of age	DR	$\bar{X}$:13.0 (11.8-14.1)	
Walker and Page (1977)	Meals from 50 colleges	Menu Analyses	(6.36-16.11) $\bar{X}$:11.03	(1.74-12.51) $\bar{X}$:3.37
White (1969)	21 women, 18-30 years $\bar{X}$:20.0	CA	(4.79-47.6) $\bar{X}$:13.8	(0.2-4.40) $\bar{X}$:0.58
White (1976)	30 college men	CA	$\bar{X}$:13.8 (4.8-47.0)	

[1] $\bar{X}$=mean, range is in brackets

REFERENCES

1. Harland, B.F., Johnson, R.D., Blenderman, E.M., Prosky, L. , Vanderveen, J.E., Reed, G.L., Forbes, A.L. and Roberts, H.R. Calcium, phosphorus, iron, iodine, and zinc in the "Total Diet". J. Am. Diet. Assoc., 1977, 16-20, 1977.

2. Harland, B.F. and Peterson, M. Nutritional status of lacto-ovo vegetarian trappist monks. J. Am. Diet. Assoc., 72, 259-264, 1978.

3. Holden, J.M., Wolf, W.R. and Mertz, W. Zinc and copper in self-selected diets. J. Am. Diet. Assoc., 75, 23-28, 1979.

4. Hunt, I.F., Murphy, N.J., Gomez, J. and Smith, J.D. Dietary zinc intake of low-income pregnant women of Mexican descent. J. Am. Clin. Nutr., 32, 1511-1518, 1979.

5. Lyon, T.D.B., Smith, H. and Smith, L.B. Zinc deficiency in the west of Scotland? A dietary intake study. J. Br. Nutrition, 42, 413-416, 1979.

6. Milne, D.B., Schnakenberg, D.D., Johnson, H.L. and Kuhl, G.L. Trace mineral intake of enlisted military personnel. J. Am. Diet. Assoc., 76, 41-45, 1980.

7. Tribble, H.M. and Scoular, F.I. Zinc metabolism of young college women on self-selected diets. J. Nutr., 52, 209-216, 1954.

8. Walker and Page, L. Nutritive content of college meals. J. Am. Diet. Assoc., 66, 146-152, 1975.

9. Walker, M.A. and Page, L. Nutritive content of college meals. II. Lipids. J. Am. Diet. Assoc., 68, 34-38, 1976.

10. Walker, M.B. and Page, L. Nutritive content of college meals III. Mineral elements. J. Am. Diet Assoc., 70, 260-66, 1977.

11. White, H.S. Inorganic elements in weighed diets of girls and young women. J. Am. Diet. Assoc., 55, 38-43, 1969.

12. White, H.S. Zinc content and the zinc-to-calorie ratio of weighed diets. J. Am. Diet. Assoc., 68, 243-245, 1976.

C.1. Nutritional, Biochemical and Pharmacological Roles, Requirements and Toxicities of Zinc and Copper

Several excellent and comprehensive reviews exist delineating the functional relevancies of zinc and copper in human, animal, and microorganism nutrition. Prasad (1979b), an early pioneer of zinc inadequacy in human diets, provides a concise review of the trace elements, zinc and copper, from a biochemical and clinical standpoint. A more detailed work by Prasad (1979a) carefully delineates the clinical, pharmacological and biochemical roles of zinc in human nutrition. A summary of the more important aspects of the two references cited above follows.

Zinc, a mineral of molecular weight of 65, is a normal constituent of body fluids, enzymes and bone. A 70 kg male contains approximately 1.5-2.0 grams of zinc, concentrated in liver, kidney, bone, retina, prostrate and muscle. Zinc in the plasma is mainly bound to albumin, but other proteins such as ceruloplasmin and a-macroglobulin may bind significant quantities. Of interest is the zinc-binding abilities of several amino acids (histidine, glutamine, threonine, cystine and lysine).

Zinc absorption, influenced by such factors as body size, phytate content and chelating agents in the diet, varies considerably in man, with approximately 20-30% of the ingestate absorbed. Animal proteins usually provide greater zinc availability than plant proteins (Prasad, 1979).

Normal zinc intake in a well-balanced diet approximates 12 to 15 mg/day.

Urinary, sweat and gastrointestinal zinc losses can be considerable (3-4 mg/day) depending, of course, on climatic or disease conditions.

The major function of zinc in biological systems centers around the 70 metalloenzymes known to require zinc as a catalyst or as part of the apoenzyme complex. As such, the metal may be found in several dehydrogenases, aldolases, peptidases, and phosphatases.

Deficiency of zinc in man occurs in a wide variety of diseased states or syndromes. A summary of the more important medical conditions is listed in Table 5.

Typical syndromes of mild zinc deficiency include growth retardation, hypogonadism in males, skin changes, poor appetite, mental lethargy and delayed wound healing. In severely zinc-deficient patients, dermatologic manifestations, diarrhea, alopecia, mental disturbances and recurrent infections predominate.

For comparative purposes, Table 6 lists some biochemical functions and manifestations of zinc and copper with their molecular weight relatives--vanadium (51), chromium (52), manganese (55), iron (55.8), cobalt (60), and nickel (58.7).

Copper has received extensive attention from reviewers in the past decade (Evans, 1973; Prasad, 1979; and Mason, 1979). The presence of copper in plant and animal tissue was first recognized almost 150 years ago. The earliest observation was the role of copper in promoting hematopoesis.

Copper is widely distributed in the body, with the highest concentrations in the liver and brain. This metal forms stable complexes and chelates with organic molecules, nucleotides, DNA, RNA, and various proteins (enzymes). Several copper-containing enzymes include cerulo-

plasmin, cytochrome oxidase, monoamine oxidase, tyrosinase, superoxide dismutase, ascorbic acid oxidase, uricase, dopamine-B-hydroxylase, galactose oxidase, and g-amino-lenulinic acid dehydrase.

Copper's ubiquitous presence in nature allows generous intakes, usually in excess of the minimum daily allowance of 2 mg. Once absorbed through the lumen of the stomach and small intestine with the aid of a copper-binding protein (intestinal metallothionein), copper binds with albumin or amino acids for transport to the liver. Ingested copper disappears rapidly from the plasma and concentrates in the liver where a secondary increase in plasma copper occurs in the form of ceruloplasmin.

As with zinc, copper levels in mammalian systems constitute wide variations due to nutritional, hormonal or pathological states. Table 7 summarizes the major metabolic conditions in which copper levels are known to play a role.

Interactions between copper and zinc have long been recognized in animals and man, as an excess of one is often associated with diminution of the other in bodily fluids and the liver. Competition for binding sites on metallothionein or metallothionein-like protein provides the best explanation. Klevay (1974) supported an hypothesis that a high zinc to copper ratio increased the risk of ischemic heart disease and associated hypercholesteremia. Although interest has been stimulated, specific human-oriented research has been lacking.

Human allowances for copper must take into account variability in absorption (40-60%), reabsorption of biliary copper and the chemical state of copper in food systems. The chelating effects of fiber, dietary phytates, sulfates and vitamin C have yet to be elucidated sufficiently. Although little is known on copper bio-availability,

states of hypocupremia in man are relatively rare, except for the high urinary losses of ceruloplasmin associated with the nephrotic syndrome in children. Paradoxically, states of hypercupremia, due mainly to high ceruloplasmin levels, are commonly observed in pregnancy, oral intake of contraceptives, and innumerable disease states and disorders.

Manifestations of copper deficiency, when they do occur, include anemia, intermittent neutropenia, severe osteoporosis and pathological fractures. Premature human infants are remarkably sensitive to poor copper intake and usually display the deficiency symptoms, described above, within two or three weeks after birth.

Copper, as compared to the other trace elements identified in Table 8, has a relatively low LD_{50}. Considering that the total body stores of copper in man approach 1 gram, an LD_{50} of 0.05 gm/kg BW (mouse) implies a narrow margin of safety in administration of copper supplements. Although small amounts of the ion (15-20 mg) may cause vomiting and diarrhea, humans and pigs are known to adapt to higher levels with rapid recovery from accidental overdoses.

Table 5. Clinical conditions leading to Zinc deficiency

Medical/Biological Condition	Etiology
1. Alcoholism: induced Hyperzincuria	Unknown. Effect on renal tubular epithelium postulated
2. Cirrhosis of liver: low serum zinc & hyperzincuria	Unknown. Zinc salts may have protective effect on rat liver
3. Renal disease: low plasma concentrations	Proteinuria and failure of tubular reabsorption
4. Dialysis: low plasma concentrations	Unknown
5. Burns and skin disorders: loss of zinc in exudates, ulcers, causing low plasma zinc	Skin contains 20% body stores of zinc, depletion through losses
6. Diabetes: Hyperzincuria	Unknown
7. Pregnancy and oral contraceptives	Zinc uptake by fetus; redistribution of zinc from plasma to RBC's
8. Acrodermatitis enteropathica: cachexia major cause of death	Zinc deficiency due to genetic malabsorption

Table 6. Biochemical functions and manifestations of essential trace element deficiency. Adapted from Bland (1979)

Element	Site of Action	Key Biochemical Function	Signs of Deficiency
Vanadium	Hemovanadin	Oxygen transport	Impaired bone & lipid metabolism (chicks, rats)
Chromium	Glucose tolerance factor	Glucose transplant	Impaired glucose metabolism
Manganese	Pyruvate carboxylase plus many enzymes	Oxidative phosphorylation and mucopolysaccharide synthesis	Defective growth, reproduction, collagen synthesis, CNS disorders
Iron	Hemoglobin (Hb) plus some enzymes	Hb synthesis	Anemia
Cobalt	Coenzyme B_{12}	Biologic Methylation	Pernicious anemia & methylmalonic aciduria
Nickel	Ribonucleic acid	Nucleic acid stabilization; membrane structure	Impaired reproduction (rats), deranged phospholipid metabolism in chicks

Table 7. Metabolic conditions affecting copper homeostasis. Adapted from Evans (1973).

Agent/Disease	Effect on Copper Concentration
Growth hormone	Depresses hepatic levels
Adrenocorticotropic hormone	Depresses ceruloplasmin and plasma copper
Addison's disease	Elevates serum copper
Corticosteroids	Reduces plasma copper
Circadium variation	Copper and ceruloplasmin's mean 24-hr. values range from a low at 1600-2400 hr. and highest at 0200-0800 hr.
Epinephrine	Elevates serum copper and ceruloplasmin
Stress	Elevates plasma copper and ceruloplasmin
Estrogens and androgens	Elevates serum copper and ceruloplasmin
Wilson's disease: (Abnormal metallothionein)	Excessive accumulation of copper in organs, low ceruloplasmin, low plasma copper
Menke's Kinky Hair Syndrome	Very low serum, hepatic, brain copper levels. (Defect in copper absorption)
Nephrotic Syndrome	Low serum copper and ceruloplasmin

Table 8. Toxicities of selected trace minerals. Adapted from Bland (1979)

Element	Action	Toxicity (LD_{50})
Zinc	Malaise, dizziness vomiting, diarrhea	2.0 gm/Kg (Rat)
Copper	Hemolytic anemia CNS degeneration	0.05 gm/Kg (Mouse)
Chromium	Cr VI more toxic than Cr III. Hyperemia, emphysema, bronchitis	0.18 gm/Kg (Rat)
Manganese	Effects CNS, cramps, tremors, hallucinations, renal degeneration	0.31 gm/Kg (Mouse)
Iron	Hemosiderosis Hemochromatosis	0.9 gm/Kg (Mouse)
Cobalt	Polycythemia, abnormal erythrocyte growth	0.5 gm/Kg (Rat)
Nickle	Dermatitis, respiratory disorders, reduces activities of Kreb's cycle enzymes	0.8 gm/Kg (Dog)

REFERENCES

1. Bland, J. Trace Elements in Human Health and Disease: II. The Physiologic Function of the Essential Trace Minerals. Osteopathic Medicine, 7, 33-43, 1979.

2. Evans, G.W. Copper Homeostasis in the Mammalian System. Physiological Reviews, 53, 535-570, 1973.

3. Mason, R.E. A Conspectus of Research on Copper Metabolism and Requirements of Man. J. Nutr., 109, 1079-2066, 1979.

4. Prasad, A.S. Clinical, Biochemical, and Pharmacological Role of Zinc. Ann. Rev. Pharmacol. Toxicol., 20, 393-426, 1979a.

5. Prasad, A.S. Trace Elements: Biochemical and Clinical Effects of Zinc and Copper. J. Am. Hematology, 6, 77-87, 1979b.

C.2. Hair, Plasma and Saliva as Indicators of Marginal Zinc/Copper Status

Symptoms of marginal zinc deficiency have been identified in several segments of the population, both in the U.S. and abroad. Typically, these symptoms include growth failure, dermatitis, intermittent hypogeusia, delayed wound healing and hypogonadism (Sandstead, 1973). Unfortunately, identifying marginally deficient subjects through biochemical and/or clinical testing remains difficult. Four of the most commonly used parameters--saliva, hair, serum, and erythrocyte zinc--seem to have their own proponents armed with viable arguments concerning the efficacy of use.

A recent focus of attention on the use of a biological fluid on the determination of marginal zinc status is saliva. The single greatest proponent of saliva usage is Henkin (1978), who proposes a mechanism for hypogeusia based on low zinc levels in the saliva with a concomitant drop in a zinc protein (gustin) alleged to have taste bud promoting properties. Henkin and his associates were first to notice the presence of zinc in saliva following labeled ^{65}Zn intravenous administration. Henkin cites many of his own studies demonstrating a reduced level of salivary zinc associated with taste loss in a variety of medical and/or metabolic disturbances (Henkin, 1975; McConnell *et al.*, 1975; Henkin *et al.*, (1976). Henkin suggests that metals, particularly zinc, appear to be involved at several levels of organization in the taste system, more specifically, the taste bud receptor. Zinc ion may even be involved in the structure of mouse 7S

Nerve Growth Factor, a compound with the ability to facilitate taste bud function (Pattison and Dunn, 1975).

Unfortunately, Henkin's enthusiasm over zinc status in saliva finds little agreement with other researchers. Everett and Apgar (1979) conducted three experiments with rats to determine whether salivary zinc concentration is a more sensitive indicator of zinc status than plasma zinc. Salivary zinc concentrations in the deficient rats did not differ from those of the zinc supplemented ad libitum-fed controls in any of the experiments. While decreases in serum, bone and fetal zinc indicated a severe zinc deficiency, salivary zinc failed to reflect these changes. The authors conclude that plasma zinc is still the best available indicator of zinc status.

Greger and Sickles (1979) collected mixed saliva from eight adolescent females participating in a metabolic study and 59 adolescent females participating in a nutrition survey. The zinc content of whole mixed saliva and the supernatant of the saliva was determined with a graphite-furnace AAS. Significantly ($p<0.05$) lower levels of zinc were found in the supernatant samples (30.5 ng/ml) than in the whole saliva samples (173 ng/ml) when the same subjects were fed 11.5 mg zinc daily rather than 14.7 mg zinc during the metabolic study. Although the levels of zinc and protein in whole mixed saliva and supernatant were correlated, the levels of zinc in both types of saliva samples were not correlated to serum or hair zinc levels of girls in the survey. The authors conclude that the use of supernatant saliva to assess zinc status may have potential. Whole mixed saliva, however, did not adequately reflect dietary status. Although Greger and Sickles (1979) expressed concern over their lack of control over flow rates, Tuompo

et al. (1977) found no correlation between salivary rate of flow and zinc levels.

Geders et al. (1980) randomly assigned 32 young women (20-40 years of age) and 31 older women (41-78 years of age) into three groups, supplementing them with 0, 15, or 50 mg zinc. After two months of zinc therapy, an increase in plasma zinc was proportional to the quantity of supplemental zinc. The young women exhibited a response that was approximately double that of the elderly women (67% vs. 37.5% increase over the initial plasma values for the 15 and 50 mg group, respectively). Maximal plasma zinc levels for the older women were attained at the 7.9 week timespan versus 5.5 for the younger women. The authors concluded that increases in the zinc content of saliva and hair in response to the two month supplementation period were not significantly different between the two age groups.

In a similar study, Buchanan et al. (1980) studied the response of zinc status parameters to zinc supplementation in older women (41-78 years). Only after the eighth week of zinc supplementation (15 and 50 mg zinc) were there significant differences in the zinc content of saliva and plasma. Significant increases in salivary zinc levels were noted one month following the 50 mg dosage. Of interest was the lack of change in hair zinc levels following zinc supplementation.

An earlier report by Baer and King (1979) monitored zinc levels in saliva, sweat, whole blood erythrocytes and feces in the experimental depletion of zinc in young men. None of these parameters demonstrated any consistent changes with depletion over nine weeks. The results suggested that the response to low dietary zinc varies greatly among

individuals. Snowden and Freeland (1979) found a circadian rhythm of zinc in saliva. Highest values of zinc were noticed in late morning, decreasing to the lowest levels in early morning. No correlations were found between the dietary intake and zinc content of saliva.

Johnson et al. (1979), in contrast to the paper by Snowden and Freeland (1979), found a decline in zinc levels of saliva after consumption of a low zinc diet (3 mg/day) for 21 days. When the subjects were given 50 mg zinc as ZN SO_4, zinc levels in saliva increased significantly, demonstrating to the authors that saliva may be a sensitive parameter for the assessment of zinc status in man. However, their levels of zinc in saliva (126 ppm) were much higher than those found by other researchers, such as Greger and Sickles (1979), who found 173 ppb.

Another concern of zinc measurements is the problem of what fraction of the whole saliva to analyze. Mathur et al. (1977) measured the relation between the zinc content of saliva and blood in healthy human adults. First, the authors noted that the mean Zn content of resting mixed saliva was one third of that in plasma and about one fifteenth that of whole blood. Secondly, the concentration of Zn in stimulated parotid saliva is only one tenth that in resting mixed saliva. In addition, nine-tenths of salivary Zn occurs in the portion of saliva containing the bulk of the mucopolysaccharide material. Finally, it is suggested that a true picture of Zn status in the body requires a knowledge of the concentrations of both Zn and Cu in the plasma.

Spencer and Samachson (1968) concur with the finding of Mathur et al. (1977). These authors found that the concentration of Zn in parotid saliva and in mixed saliva varied greatly in the different

patients. Not only was there great variability in the Zn levels in replicate samples of parotid and mixed saliva throughout the day, but the concentration of Zn in mixed saliva was consistently higher than in the parotid sample. The authors note that magnesium and calcium were found to have relatively constant concentrations in different samples of saliva obtained from the same person.

Hair and Zinc/Copper Nutritional Status

The question of using hair biopsies to assess marginal zinc/copper status is as controversial as the use of saliva. Of greater importance, however, is the question of whether hair mineral analysis reflects dietary intake of the same minerals.

In a most recent review of zinc status and elderly Black Americans,(Wagner et al. 1980) summarized the literature concerning the efficacy of hair samplings. In the elderly, at least, the available information suggests that hair may be a useful index of zinc nutriture (Greger and Sciscoe, 1977; Greger, 1977; Greger and Geissler, 1978). In other individuals, however, the use of hair for the evaluation of human zinc nutriture remains somewhat controversial (Solomon, 1979; Hilderbrand and White, 1974; McKenzie, 1978). These authors suggest that contamination from exogenous sources, beauty treatments, and variable hair growth rates may complicate the interpretation of hair zinc data. Other evidence from animal studies has demonstrated that hair zinc concentrations reflect chronic or long term zinc nutriture and that it correlates with the zinc content of bone and diet (Reinhold et al., 1968; Lewis et al., 1957). Depressed hair zinc levels in children and adults have also been associated with human zinc

deficiency (Hambridge et al., 1972, Strain et al.,1966).

Flynn et al. (1971) have emphasized that hair can be of great usefulness in nutritional assessment, if attention is given to proper sampling techniques and appropriate statistical analyses. Researchers generally agree that homogeneity in the distance of the sample from the scalp is an important consideration and that only that portion of the hair closest to the scalp should be sampled for zinc analysis. This newly grown hair is presumably more reflective of current metabolic status and is less subject to contamination from exogenous sources.

The measurement of serum or plasma zinc is also frought with difficulties in interpretation and biological relevance. Although a depressed concentration of circulating zinc in plasma or serum has been associated with human zinc deficiency (Hambidge et al., 1976; Halsted et al., 1974; Baer and King, 1978; Prasad et al., 1978), circulating zinc levels are not always a reliable index of zinc nutriture (Mertz, 1975; Solomons, 1979; Baer, et al., 1978). Numerous factors which are known to affect zinc status include: hemolysis, hypoproteinemia, hyperzincemia, infection, weight loss, and disease states. In addition, it should be pointed out that normal plasma zinc levels do not necessarily imply adequate body stores of this nutrient, because in some individuals plasma zinc values remain in the normal range even when the skin lesions of zinc deficiency are apparent (Baer et al., 1978).

The variety of studies on zinc nutriture and the relevance of hair biopsies in its assessment are listed in Table 9.

TABLE 9. Efficacy of hair serum sampling in the assessment of mineral deficiences, particularly zinc and copper.

AUTHOR	TITLE (SUBJECTS)	HAIR Zn/Cu	SERUM Zn/Cu	COMMENTS
Assarian and Oberleas (1977)	Effect of washing procedures of trace-element content of hair.	Authors studied three methods of hair preparations--a detergent wash, a hexane-ethanol wash, and acetone-ether-detergent wash. Results indicate that elements in hair are sensitive to the preparation technique and therefore are unreliable for information on trace-element status.		
Briggs et al. (1971)	Trace elements in human hair (many classifications of human subjects)	Cu: 18 ppm. Zn: 205 ppm.		Individual variations are large, and it is doubtful whether trace elements in hair could be a diagnostic aid.
Deeming and Weber (1977)	Hair zinc status in rats.	Zn: 210 ppm.		Plasma Zn did not change with diet. Hair Zn is useful as an aid to Zn deficiency for the rat, but not to assess the state of zinc metabolism.
Deeming and Weber (1978)	Trace elements and age, sex, and drugs. (Subjects 16 to 84 years, 11 male and 16 female).	Zn: Males: 176 ppm. Females: 208 ppm. Cu: Males: 23 ppm. Females: 47 ppm.	Zn: Males: 182 Females:165 Cu: Males: 196 Females:270 All values in ug/100 ml.	Hair Zn decreased with age. Hair minerals did not correlate well with either serum levels or dietary intake.

TABLE 9. (continued)

AUTHOR	TITLE (SUBJECTS)	HAIR Zn/Cu	SERUM Zn/Cu	COMMENTS
Epstein et al. (1980)	Hair copper in biliary cirrhosis. (11 females, 50 to 61 years).	Cu: range of 7-23 ppm.		There was no correlation among liver, copper, plasma copper or ceruloplasmin concentrations.
Erton et al. (1978)	Hair zinc in healthy and malnourished children (MC) (115 subjects, 0 to 15 years).	F/M 0-5 yrs: 133/119 ppm. 6-10 : 143/168 ppm. 10-15 : 166/192 ppm. MC: 170.5 ppm. with a range of 78-276.	MC: 64.5 ug/ 100 ml.	Levels of hair Zn increase with age. MC had higher hair Zn levels with normal subjects. No correlation of hair zinc and serum.
Gershoff et al. (1977)	Trace minerals in human and rat hair (430 boys and girls, 1-1/2 to 7 years).	Zn: Males: 115 ppm. Females: 80.0 Cu: Males: 20 Females: 16 ppm.		Hair levels of Zn/Cu are of little value in predicting health status. Rat hair levels of Zn/Cu greatly varied with identical diets.
Hambidge (1973)	Hair copper and distance from scalp. (27 subjects, 4 to 32 years, x =14).	Cu: 71.8 ppm. for distal portions: 20.7 ppm.		Mean hair Cu varies with scalp distance. Sample hair within 1 to 2 cm of scalp.
Jacob et al. (1978)	Copper and zinc in hair as an index of hepatic metal in rats.	Cu: 9.4 ppm. Zn: 198 ppm.		Mean hair metals paralleled the dietary intakes for copper, not zinc. Copper in hair correlated with liver copper.

TABLE 9. (continued)

AUTHOR	TITLE (SUBJECTS)	HAIR Zn/Cu	SERUM Zn/Cu	COMMENTS
Krebs et al. (1980)	Hair zinc in retarded children.	Zn: from 96 ppm. 138 ppm. Wide range in values.		Dietary analysis of 24 hour recalls. Showed no significant correlation between zinc intake and hair levels.
Klevay (1970)	Hair as a biopsy material (copper) (children and adults).	Cu: Males:22.4 ug/g Females:39.3 ug/g	Cu: Males: Plasma : 141 Females: 139 Cu: males: RBC: 127, Females: 104	Copper in hair varies with age and sex. Female adults have more Cu than males. Positive correlation between hair copper and plasma, and RBC Cu.
Klevay (1970)b.	Hair as biopsy material. (Subjects aged 1 to 83 years).	(all in ppm.) Means: 147, 127, 126, 163, 142. for ages 0-5, 6-10, 11-15, 16-20, 20. (for males)	Plasma in ug/100 dl:125. RBC's in mg/100g:0.968.	Zn in RBC/Zn in plasma is a 10:1 ratio. No correlation among plasma, hair and RBC Zn values. Zn in hair is useful indicator.
Lindman (1971)	Age/sex and Plasma and RBC zinc levels (204 males, 20 to 84 years; 54 females, 20 to 58 years).		Plasma for males: 96 ug/dl For female: 88 ug/dl RBC for males: 15.7 ug/cc, females: 15.8 ug/cc	Plasma Zn decreases with age. RBC Zn had no change with age or sex.
Murthy et al. (1974)	Zinc/Copper nutriture in the rat.	Zn: initial values: 159.3 ppm. Cu: initial values: 41.3 ppm.		Hair is a good biopsy material for Zn nutriture, but copper wasn't. Inverse "R" between hair Cu and serum Zn. Hair Zn directly related to serum zinc.

TABLE 9. (continued)

AUTHOR	TITLE (SUBJECTS)	HAIR Zn/Cu	SERUM Zn/Cu	COMMENTS
Petering et al. (1971)	Trace metal content of hair; Zn/Cu (211 persons, 1 to 80 years).	Males: age 2:105 ppm. to 280 ppm. at age 12 to 125 ppm. at age 80. Females very similar.	Hair copper: 13 ppm at age 2, 60 ppm at age 12 then 10 ppm. at age 80. (Males)	Females showed increase in copper with age, and a decrease in Zn. Males decreased both with age.
Reinhold et al. (1966)	Zn/Cu in hair of Iranians.	Villagers: Zn: 127.0 ppm Control: 220 ppm. Cu: 12.0 Controls: 13.3 ppm.		Zn depleted in Iranian villagers, but not copper. Hair reflects diets presumably.
Reinhold et al. (1967)	Zn/Cu in hair with low zinc and protein intakes in rats.	Zn: @180 ppm. Cu: 13.5 ppm.		No "R" between zinc intake and Cu levels of hair. Zn in hair and diet are related.
Reinhold et al. (1968)	Zinc nutrition and hair levels in rats.	@ 179 ppm. for controls. @ 115 ppm. for Zn deficient.		Zn in hair depends on intake, but may not reflect Zn deficiency as manifested by impaired growth rates.
Solomons et al. (1976)	Zinc nutrition and celiac sprue.		Plasma Zn: 51.5 ug/100 ml. Plasma copper: 102.6 ug/100 ml.	Depressed plasma Zn and copper in celiac sprue.

TABLE 9 (continued)

AUTHOR	TITLE (SUBJECTS)	HAIR Zn/Cu	SERUM Zn/Cu	COMMENTS
Strain et al. (1966)	Zinc deficiency and zinc in hair (normal Egyptians: NE, dwarfs: D).	NE: 103.3±4.4 ppm. D: 54.1 ppm.		Highest Zn in summer months. Oral $ZnSO_4$ increased hair Zn in D to 121 ppm. Hair analysis is reliable, simple, and atraumatic.
Vir and Love (1979)	Zn/Cu status in the elderly (146 subjects, 65 to 95 years of age).	203.1 to 246.6 ug/g Zn. For Cu: 20 ug/g with a range: 16.4 to 30.4	72.3 to 84.6 ug/100 ml for Zn. For Cu: 112 ug/100 ml.	Hair cations had no "R" with age or sex. Plasma and hair did not correlate. Plasma Zn and albumin did correlate.
Wagner (1980)	Zinc status of Black Americans aged 60 to 87 years.	142±77 ug/g 39% had hair zinc <100 ug/g.	93±15 ug/dl 39% had 70 ug/g.	Elderly Zn status is less than ideal. Hair is useful index of Zn.

REFERENCES

1. Assarian, G.S. and Oberleas, D., Effect of washing procedures on trace-element content of hair. Clin. Chem., 23/9, 1771-1772, 1977.

2. Briggs, M.H., Briggs, M. and Wakatama, A., Trace elements in human hair. Experientia, 28, 406-407, 1971.

3. Deeming, S.B. and Weber, C.W., Evaluation of hair analysis for determination of zinc status using rats. J. Am. Clin. Nutr., 30, 2047-2052, 1977.

4. Deeming, S.B. and Weber, C.W., Hair analysis of trace minerals in human subjects as influenced by age, sex, and contraceptive drugs. J. Am.Clin.Nutr., 31, 1175-1180, 1978.

5. Epstein, O., Boss, M.A., Lyon, D.B. and Sherlock, S., Hair copper in primary biliary cirrhosis. J. Am.Clin.Nutr., 33, 975-956, 1980.

6. Erten, J., Arcasoy, A., Cavadar, A.O. and Cin, S., Hair zinc levels in healthy and malnourished children. J. Am. Clin. Nutr., 31, 1172-1174, 1978.

7. Geders, J., Freeland-Graves, J., King, J., and Buchanan, D., Difference in response to zinc supplementation in young and older women. Federation Proceedings, 37, 2048, 1980.

8. Gershoff, S.N., McGandy, R.B., Nondasuta, A., Pisolyabutra, V. and Tantiwongse, P., Nutrition studies in Thailand III. Trace minerals in human and rat hair. J. Am. Clin. Nutr., 30, 868-872, 1977.

9. Hambidge, K.M., Increase in hair copper concentration with increasing distance from the scalp. J. Am. Clin. Nutr., 26, 1212-1215, 1973.

10. Jacob, R.A., Levay, L.M. and Logan, G.M., Hair as a biopsy material V. Hair metal as an index of hepatic metal in rats; copper and zinc. J. Am. Clin. Nutr., 31, 477-480, 1978.

11. Klevay, L.M., Hair as a biopsy material II. Assessment of copper nutriture. J. Am. Clin. Nutr., 23, 1194-1202, 1970.

12. Klevay, L.M., Hair as a biopsy material. I. Assessment of zinc nutriture. J. Am. Clin. Nutr., 23, 284-289, 1970.

13. Krebs, N.F., Moser, D.P., Blyter, E.M., Smith, J.C. and Kozlowski, B.W., Hair zinc concentrations in small-for-age and normal sized functionally retarded children. Fed. Proc., Ant., 2045, 1980.

14. Lindeman, R.D., Clark, M.L. and Colmore, J.P., Influence of age and sex on plasma and red-cell zinc concentrations. J. Geront., 26, 358-363, 1971.

15. Mathur, A., Wallenius, K. and Abdulla, M., Relation between zinc content in saliva and blood in healthy human adults. Scand. J. Clin. Lab. Invest., 37, 469-472, 1977.

16. Murthy, L., Klevay, L.M. and Petering, H.G., Interrelationships of zinc and copper nutriture in the rat. J. Nutr., 104, 1458-1465, 1974.

17. Petering, H.G., Yaeger, D.W. and Witherup, S.O., Trace metal content of hair I. Zinc and copper content of human hair in relation to age and sex. Arch. Environ. Health, 23, 202-207, 1971.

18. Reinhold, J.G., Kfoury, G.A., Ghalambor, M.A. and Bennett, J.C., Zinc and copper concentrations in hair of Iranian villagers. J. Am. Clin. Nutr., 18, 294-300, 1966.

19. Reinhold, J.G., Kfoury, G.A. and Thomas, T.A., Zinc, copper and iron concentrations in hair and other tissues: effects of low zinc and low protein intakes in rats. J. Nutr., 92, 173-182, 1967.

20. Reinhold, J.G., Kfoury, G.A. and Arsuanian, M., Relation of zinc and calcium concentrations in hair to zinc nutrition in rats. J. Nutr., 96, 519-524, 1968.

21. Spencer, H. and Samachson, J., Secretion of alkaline, earth metals in saliva. In: "Secretory Machanisms of Salivary Glands." Schmeyer and Schmeyer, EDS., 101-113, 1968.

22. Solomons, N.W., Rosenberg, I.H. and Sandstead, H.A., Zinc nutrition in celiac sprue. J. Am. Clin. Nutr., 29, 371-375, 1976.

23. Solomons, N.W., On the assessment of zinc and copper nutriture in man. J. Am. Clin. Nutr., 32, 856-871, 1979.

24. Strain W.H., Steadman, L.T., Lankau, C.A., Berlinger, W.P. and Pories, W.J., Analysis of zinc levels in hair for the diagnosis of zinc deficiency in man. J. Lab. Clin. Med., 68, 244-249, 1966.

25. Vir, S.C. and Love, A.H.G., Zinc and copper status of the elderly. J. Am. Clin. Nutr., 32, 1472-1476, 1979.

26. Wagner, P.A., Krista, M.L., Bailey, L.B., Christako, G.J., Jernigan, J.A., Araujos, P.E., Appledorf, H., Davis, C.G. and Dinning, J.S., Zinc status of elderly black americans from urban low-income households. J. Am. Clin. Nutr., 33, 1771-1777, 1980.

C.3. Factors Influencing Zinc/Copper Absorption from Supplements

Although many researchers have used oral zinc/copper supplements to alleviate clinical and/or nutritional disorders, the actual biochemics of absorption, as influenced by dietary foodstuffs, remains obscure.

Ingestion of zinc or copper salts on an empty stomach precipitates gastric irritation, intestinal motility changes, and, in some cases, diarrhea. Although acceptance of oral doses of the metals is enhanced after a meal, absorption is not. The negative influence of breads rich in phytate, interference of copper, and interactions with other nutrients can have profound effects on serum zinc levels. In one study on the effect of foodstuffs on the absorption of zinc sulfate, Pecoud (1975) demonstrated decreased zinc absorption when subjects consumed dairy products and brown bread. When 50 mg of zinc were given with coffee, the serum zinc concentration was much lower than the equivalent dose with water. When purified phytate, phosphate and calcium were given with 50 mg of zinc, only the addition of 500 mg calcium did not induce consistently lower serum levels. Of special interest was the report of gastric discomfort after 50 mg zinc on an empty stomach in five of the six subjects. The authors concluded with the warning that the absorption of $ZnSO_4$ may be completely inhibited when the drug is given with meals containing dairy products.

In another study of similar experimental design, Andersson et al. (1976) studied serum zinc levels in eight volunteers after oral administration of 50 mg zinc as zinc sulfate. Maximum serum levels

were obtained three hours after ingestion. Some of the healthy subjects and some of the patients reported slight gastrointestinal discomfort within 30-60 min. after swallowing the zinc sulfate. Since there was little difference between the concentrations of zinc in portal and peripheral venous blood during absorption, the authors suggested the slow passage of zinc across the intestinal wall inhibits zinc absorption.

A more recent article by Oelshlegel and Brewer (1977) studied the absorption of pharmacologic doses of zinc in more detail. In one study which followed the serum zinc levels of nine volunteers over one year, considerable variability was encountered; consistently high and low levels were noticed. This same variability on elevated serum zinc values was evident upon administration of 110 mg zinc sulfate in six volunteers. As expected, plasma zinc values vary as a function of dosage, with the highest serum values obtained three hours after ingestion of 50, 25, and 12.5 mg Zn.

Because zinc sulfate may not be well tolerated, the authors compared the efficacy of using two zinc salt alternates, zinc acetate and zinc carbonate. In a serum comparison of zinc levels, the sulfate and acetate compounds showed very similar zinc tolerance curves, while the zinc carbonate exhibited lower plasma zinc levels after three hours.

The final study compared serum zinc levels after consumption of various foodstuffs. In subjects consuming 25 mg Zn, the ingestion of a single hamburger bun had adverse effects on serum levels. More importantly, many food items--celery, lemon juices, coffee, hard-boiled eggs, and milk---had marked inhibitory effects on zinc absorption. Meat, histidine, glutamine, cola, club soda, ginger ale, tea, and apples did not appear to affect zinc levels.

Two very recent reports add additional caveats to the proper use of oral zinc supplements. Casey et al. (1980) studied the variations in zinc absorption in subjects consuming a standard breakfast, infant milk formulas or Lofenalac[R]. The standard breakfast of white toast, butter and jam was ingested with 25 mg zinc. Plasma zinc was determined prior to and at 30 minute intervals following ingestion. Although wide variability occurred in peak serum zinc values, highest values were obtained after 60-90 minutes. Male control subjects exhibited consistently lower zinc uptake values than the females, in spite of the addition of picolinic acid or citrate designed to increase absorption. Lofenalac[R] significantly decreased zinc absorption, which may explain the poor zinc nutritional status of many children with PKU.

In the other study of the intestinal interaction of zinc and iron in man, Solomons and Jacob (1980) concluded that nonheme but not heme iron competitively inhibits the intestinal absorption of zinc in man. Fe/Zn ratios of 2:1 and 3:1, but not 1:1, resulted in progressive inhibition of zinc absorption. The authors found a Fe/Zn ratio greater than 3:1 in 21 vitamin-mineral supplements listed in the Physician's Desk Reference (1977) which suggests intrinsic interference with the biological availability of zinc.

The decreased absorption of zinc after administration of a Guatemalan Rural Diet was observed by Solomons et al. (1979). After administration of 25 mg zinc, serum values rose significantly the first hour. Ingestion of tortillas, bean gruel, sweet rolls and black coffee with the zinc administration significantly lowered plasma zinc. The authors suggested that dietary phytates, fiber and calcium are the prime considerations for the observed inhibition of zinc absorption. Tsai and Lei (1979) demonstrated that even with high levels of

cellulose in the diet (16%), the whole body homeostasis toward zinc and copper was only slightly affected. Although increasing amounts of cellulose in the diet decreased serum zinc levels by a small amount, no detrimental effect on the distribution of zinc, iron, or copper in tissues was noted.

The ultimate control of metal absorption apparently resides in the small intestine, at least in the rat biosystem. Evans et al. (1979), using radioisotope dilution to follow zinc absorption in rats, found that zinc homeostasis in rats is maintained by zinc secretion from the intestine rather than by regulation of zinc absorption. In addition, mature rats absorbed as much or more zinc than young rats fed the same dietary level of zinc. These surprising results were also encountered by Weigand and Kirchgessner (1976) who found that rats absorb far more than the daily zinc requirement and maintain zinc balance by secretion of zinc in the intestine with eventual elimination in the feces. Evans et al. (1979) even goes so far as to suggest his earlier interpretation of zinc absorption was incorrect. That is, rats fed a zinc deficient diet do not absorb more zinc than rats fed a zinc adequate diet (Evans et al., 1975). Mature rats simply secrete more endogenous zinc into the intestine than do young growing rats. By studying the net uptake of zinc from segments of rat duodenum, jejunum and ileum using in vivo intestinal perfusions, the authors suggest that the capacity for zinc absorption in the rat is significantly greater in the ileum (60.1%), at least in in vivo perfusions. Another finding of interest was the observation of rapid zinc transport across the intestinal lining upon contact with zinc.

It is apparent that many food items can negatively influence zinc absorption, and presumably, copper absorption when taken in the form of supplements. Therefore, the design of zinc/copper ingestion protocols or therapies must consider the types and timings of foodstuffs taken along with zinc. Armed with the foregoing information one may conclude:

1. Zinc preparations should be given after the meal is completed, preferably 30-60 minutes.
2. Zinc and copper, and possibly other minerals, should not be given together to minimize competitive absorption. However, since there is a delicate balance in the zn/cu ratio of the blood, a disproportional amount of zinc absorbed relative to that of copper may initiate or exacerbate nutritional and/or medical disorders.
3. As compared to zinc sulfate, zinc acetate or another conjugate may lessen gastric irritation (Oelshlegal and Brewer, 1977).

REFERENCES

1. Andersson, K.E., Bratt, L., Dencker, H., and Lanner, E, Some aspects of the intestinal absorption of zinc in man. Europ. J. Clin. Pharmacol., 9, 423-428, 1976.

2. Antonson, D.L., Barak, A.J., and Vanderhoof, J.A., Determination of the site of zinc absorption in rat small intestine. J. Nutr., 109, 142-147, 1979.

3. Casey, C.E., Walravens, P.A., and Hambidge, K.M., Variations in zinc absorption. Federation Proceedings 39, p. 1213, Abstract 2041, 1980.

4. Evans, G.W., Johnson, E.C., and Johnson, R.E., Zinc absorption in the rat determined by radioisotope dilution. J. Nutr. 109, 1258-1264, 1979.

5. Hahn, C.J., and Evans, G.W., Absorption of trace metals in the zinc-deficient rat. J. Am. Physiology 228, 1020-1023, 1975.

6. Oelshlegal, Jr., F.J., and Brewer, G.J., Absorption of Pharmacologic doses of zinc. In: "Zinc metabolism: Current aspects in health and disease," Brewer, G.J. and Prasad, A.S., EDS., Alan R. Liss, Inc., New York, pp. 299-311, 1977.

7. Pecoud, A., Donsel, D., and Schelling, J.L., Effect of foodstuffs on the absorption of zinc sulfate. Clin. Pharmacol. Ther. 17, 469-474, 1975.

8. Physician's Desk Reference., Huff, B., Editor, Medical Economics Company, New Jersey, 1977.

9. Solomons, N.W., Jacob, R.A., Pineda, O., and Viteri, F.E., Studies on the bioavailability of zinc in man. Effects of the Guatemalan rural diet and of the iron-fortifying agent, NaFe EDTA. J. Nutr. 109, 1519-1928, 1979.

10. Solomons, N.W., and Jacob, R.A., Studies on the intestinal interaction of zinc and iron in man. Federation Proceedings, 39, 430, 1980 (Abstract 856), 1980.

11. Tsai, R.C.Y., and Lei, K.Y., Dietary cellulose, zinc and copper: effects on tissue levels of trace minerals in the rat. J. Nutr., 109, 1117-1122, 1979.

D.1. Animal Studies of Altered Feeding Behavior(s) with Zinc or Copper Deficient Diets.

Studies concerning the influence of zinc or copper deficient diets on taste preferences in animal model systems provide more definitive information on possible taste-mineral interactions than clinical studies. Several investigators studied the effect of a zinc deficient diet on fluid intakes, altered preferences for sodium chloride and changes in plasma and urinary zinc concentrations (Chesters and Quarterman, 1970; McConnell and Henkin, 1974; Catalanotto and Lacy, 1977; Catalanotto, 1979).

The effects of a zinc deficient diet on food intake and feeding patterns of rats were studied by Chesters and Quarterman (1970). Rats fed the Zn deficient diet reduced voluntary food intake to 70% of the control level. In addition, the Zn deficient rats ceased to gain weight and increased their day-to-day variation of feed intakes. The addition of microgram quantities of $ZnSO_4$ to the Zn deficient rat diets produced large increases in food intake within four hours. Finally, Zn deficient rats ate more of test diet containing 6 ppm Zn as compared to the 1 ppm diet. Zn supplemented diet preference was also noted by Gordon et al. (1980) who showed that Zn deficient rats (<2 ppm) ate significantly more of the Zn supplemented diet, whereas the zinc supplemented rats (50 ppm) and zinc supplemented ad libitum rats did not.

In another early experiment, McConnell and Henkin (1974) observed changes in NaCl ingestion patterns, plasma and urinary zinc in rats fed zinc deficient or zinc supplemented diets ad libitum. NaCl preference was significantly greater in zinc deficient rats; this

alteration in preference occurred within three days of the zinc deficient diet initiation, although anorexia set in the next two days. Concentrations of plasma and urinary zinc were significantly lower in rats fed the zinc deficient diet than pair-fed rats or rats fed the zinc supplemented diet ad libitum.

In a similar study, Catalanotto and Lacy (1977) demonstrated an increased intake of NaCl solutions in zinc deficient rats, compared to normal rats, presumably due to decreased taste acuity. In addition to NaCl intake, the authors studied two-bottle preference for sucrose, quinine sulfate (QS) and hydrochloric acid (HCL) solutions, since NaCl intake may be reflective of serious pathophysiologic processes not related to taste. One group was pair-fed a zinc deficient diet supplemented with $ZnSO_4$, 100 ppm, while the other group received the zinc deficient diet, ad libitum. Compared to the controls, the deficient rats displayed significantly increased mean preferences for the sucrose, quinine and HCL solutions.

In a subsequent report, Catalanotto (1979) designed an experiment to control for postingestional determinants upon fluid consumption utilizing a two-bottle, one hour test combined with a restricted three hour eating period. Zinc deficient rats demonstrated significantly greater preference for all the tastant-containing solutions (0.15 m NaCl; 0.0025 m HCL; 0.00000128 m QS; and 0.30 m NaCl and 0.00000128 m QS), but showed both significant increases and decreases in total volume intakes. The authors suggest that increased preferences of zinc deficient rats may be related to preingestional, i.e., taste, rather than postingestional cues.

Increased preferences by zinc deficient animals to tastant-containing solutions suggest a physiological aberration or alteration in the gustatory mechanism(s). Catalanotto (1978) hypothesized that if the hyperkeratosis, which was observed by other investigators (Usmanski and Meyer, 1969), extended over the fungiform and circumvallate papilla, the pores of the taste buds might become blocked and prevent the interaction of tastant and receptor. Subsequent histological studies (Catalanotto and Nanda, 1978) failed to confirm the hypothesis, however. While the taste buds appeared smaller, the area of the pore surface exhibited a relatively normal layer of keratotic material similar to that seen in the control animals. Although the taste buds of zinc deficient animals seemed altered, cellular differences were difficult to determine.

Chaudhry and Meyer (1979) noted the failure of Catalanotto's taste pore hypothesis to explain zinc deficiency and taste impairment. They suggested, however, an hypothesis of their own. Functional impairment of salivary myoepithelial cells, the authors suggest, present in zinc deficient animals, might result in an impaired flow of saliva to the taste cells. Assuming that saliva contains the postulated trophic factor of Henkin et al.(1975), then interrupted flow rates or reduced volumes of saliva may impair normal taste cell development. Copper, in addition to zinc, may be involved in the gustatory mechanisms as demonstrated by taste preference studies and copper depletions in rats.

An early study by Kare and Henkin (1969) involved the use of D-Penicillamine (D-Pen) added to the diet of rats, a drug which, at least in humans, causes noticeable hypogeusia. D-Pen, added to the

diet at a concentration of 2 g/kg, caused significant increases in preference for 0.15 and 0.3 M NaCl solutions. The authors suggested the altered taste acuity may be due to biochemical alterations in copper and/or thiol metabolism.

In another study, Catalanotto and Henkin (1972) elaborated on the effects of D-Pen and cysteine on NaCl preference and copper and zinc metabolism in the rat. Rats fed D-Pen exhibited greater intakes for all NaCl solutions, decreased serum copper and increased urinary copper and zinc excretions. Rats fed cysteine exhibited higher intakes for 0.15-.60 M NaCl solutions, but had normal serum and urinary zinc/copper concentrations. The results indicated that D-Pen can suppress taste acuity to an extreme degree and produce anemia and endocrine malfunction, perhaps through copper depletion. Dietary intake of thiol alone (cysteine) might also suppress taste acuity through thiol group binding to the taste bud membrane.

Zawalich (1971) also found marked preference shifts in D-Pen treated rats, but concluded that since electrophysiological and behavioral thresholds for NaCl were not altered, D-Pen cannot work by changing the peripheral sensitivity of the taste bud. Zawalich suggested a mechanism of action in D-Pen treated animals that centered around the possible chelating action of the drug to copper stores in the CNS. The author conceded, however, that there has yet to be established a simple relationship between sensitive or integrated nervous activity and preference.

Yamamoto and Kawamura (1971) demonstrated, using electrophysiological studies of chemoreception, a general inhibition in the sweet taste response upon application of 0.001 and 0.0001 M copper and zinc.

ions to a rat tongue. The sweet taste, elicited by 1M sucrose solutions, was depressed (magnitude of integrated chorda tympani response) 10 to 30% of the control response. The inhibitory effect of cupric ions was stronger than that of zinc ions in the same concentrations. Human saliva normally contains 10^{-6}M zinc and 10^{-7}M copper, most of it being bound, however, and not in the ionized form (Dreizen et al., (1970). The authors concluded by noting a difference in the inhibitory mechanisms of the ions; cupric ion competes with the sugar molecule for the same receptor site, while the zinc ion acts non-competitively.

Elaborating on their earlier observations, Yamamoto et al., (1978) studied taste preference and chorda tympani nerve responses of rats under copper toxicosis. Injection of 450 ug $CuCl_2$/100 g BW for two weeks increased preference for tartaric acid and quinine solutions, but not for NaCl or sucrose solutions. Discontinuation of copper administration restored the altered preferences to the preference level of the control period. Summated taste nerve response to varying concentrations of the four basic taste stimuli in the copper-injected rats was similar to those in the control rats. Several possible mechanisms may exist to explain the observed changes in preference behavior. First, the lack of chorda tympani changes to copper injection does not exclude peripheral changes that might be present in other gustatory constituents. Second, single taste fibers in copper-injected rats may have changed without any manifestation of altered whole nerve activity. Third, copper may exert its effect indirectly by influencing enzyme activities or other biological catalysts.

Catalanotto (1978) best summarized the influence of metal ions (notably zinc) on taste preference:

> "The animal studies with various drugs seem to indicate that thiols as well as agents that deplete trace metals such as zinc cause an increased intake of certain solutions distinguished primarily by their taste. We interpret this to mean a change in taste funtion. . . Animal studies with a dietary induced zinc deficiency . . . cause an increased intake of tastant-containing solutions and we again interpret this as modified taste function. . . Depletion of zinc can lead to decreased taste acuity but decreased taste acuity is not necessarily associated with depletion of zinc."

REFERENCES

1. Catalanotto, F.A., and Henkin, R.I., Effects of thiols on sodium chloride preference and copper and zinc metabolism in the rat. Am. J. of Physio., 222, 1594-1598, 1972.

2. Catalanotto, F.S., and Lacy, P., Effects of a zinc deficient diet upon fluid intake in the rate. J. Nutr., 107, 436-442, 1977.

3. Catalanotto, F.A., and Nanda, R., The effects of administration of a zinc deficient diet on taste acuity and tongue epithelium. J. Oral Pathol., 6, 211-217-, 1978.

4. Catalanotto, F.A., The trace metal zinc and taste. J. Am. Clin. Nutr., 31, 1098-1103, 1978.

5. Catalanotto, F.A., Alterations of short-term tastant-containing fluid intake in zinc deficient adult rats. J. Nutr., 109, 1079-1085, 1979.

6. Chaudhry, I.M., and Meyer, J., Response of submandibular gland of the rat to nutritional zinc deficiency. J. Nutr., 109, 316-320, 1979.

7. Chesters, J.K., and Quarterman, J., Effects of zinc deficiency on food intake and feeding patterns of rats. J. Nutr., 24, 1061-1069, 1970.

8. Henkin, R.K., Lippoldt, R.E., Bilstad, J., and Edelhoch, H., A zinc protein isolated from human parotid saliva. Proc. Nat. Acad. Sci. U.S.A., 72, 448-494, 1975.

9. Gordon, E.F., Denny, M.R., and Bond, J.T., Behavioral and physiological effects of experimental zinc deficiency in young and aging rats. Federation Proceedings, 39, #1, p. 431, 1980.

10. Kare, M.R., and Henkin, R.I., The effects of D-Penicillamine on taste preferences and volume intake of sodium chloride by the rat (33925). Proc. Soc. Exptl. Biol. Med., 131, pp. 559-565, 1969.

11. McConnell, S.D., and Henkin, R.I., Altered preference for sodium chloride, anorexia, and changes in plasma and urinary zinc in rats fed on zinc-deficient diet. J. Nutr., 104, 1108-1114, 1974.

12. Osmanski, C.P., and Meyer, J., Ultrastructural changes in buccal and palatal mucosa of zinc deficient rats. J. Invest. Dermatol., 53, 14-23, 1969.

13. Tamamoto, T., and Kawamura, Y., Inhibiting effect of cupric and zinc ions on sweet taste response in the rat. J. Osaka Univ. Dent. Sch., 11, 99-104, 1971.

14. Yamamoto, T., Kosugi, T., and Kawamura, Y., Taste preference and taste nerve responses of rats under copper toxicosis. Pharmac. Biochem. and Behav., 9, 799-807, 1978.

15. Zawalich, W.S., Gustatory nerve discharge and preference behavior of penicillamine treated rats. Physiology and Behavior, 6, 419-423, 1971.

D.2. Zinc/Copper Supplementation in the Chemical Senses

The General Population

An association between trace metals and gustatory and/or olfactory function has been suggested by several authors for some time. Approximately 80% of all such mineral supplementation studies, with the associated sensory restorations, have been completed by Henkin or his colleagues. Although recent reviews by Catalanotto (1978) and Anonymous (1979) tend to minimize the importance of Henkin's work, the great majority of his work, that showed improvement in taste/smell function, cannot be categorically ignored by serious researchers.

Hence, the reader is invited to come to his own conclusions by perusal of the following papers listed in Table 10. At present, the opinion of this author agrees with the observation by Catalanotto:

> "Depletion of zinc can lead to decreased taste acuity but decreased taste acuity is not necessarily associated with depletion of zinc."

Table 10. A General Survey of Trace Metal Metabolism in Sensory Evaluation of Taste and Smell.[1]

Author	Type of Study	Subjects	Biological Measurements	Sensory Measurements	Conclusions
Atkin-Thor et al.	Hypogeusia in chronic dialysis patients. (CDP).	20 CDP aged 21-70 years.	Serum Zn. Calorie intake. Hair Zn. Note: serum Zn showed wide variations.	Double-blind cross-over 440 mg $ZnSO_4$, 3 x week for 6 weeks. Side effects of supp.: nausea, dizziness, malaise, dis-equilibrium.	Tastes improved after Zn therapy. Appetite improved, food calorie intake inc., hair Zn inc. (115 to 184 ppm). serum Zn went (99 to 138 ug/100 ml).
Burch et al. (1974)	Gustatory and olfactory acuity in cirrhosis.	8 male patients 7 control subjects.	Biopsy proven cirrhosis. serum Zn, Cu, mg, ca, mn, & selenium.	DT and RT of basic tastes and smell by Henkin's methods.	Each patient had lower DT &RT's for taste and smell in at least one taste substance. Patients exhibited anorexia, hypogeusia and hyposmia.
Burge et al. (1979)	Taste acuity and hemodialysis.	18 patients (17-65 years) with end-stage kidney disease. 10 controls.	Creatinine, urea, serum Zn and hematocrit.	RT (Quinine, tartaric acid, NaCl, sucrose)- After 2 correct identifications.	Hemodialysis improved RT's, with no change in serum zinc (77 ug/dl). Sweet & sour showed the greatest change pre and post dialysis.

[1] DT: Detection Thresholds; RT: Recognition Thresholds.

Table 10, (continued)

Author	Type of Study	Subjects	Biological Measurements	Sensory Measurements	Conclusions
Casper et al. (1980)	Trace metals, vitamins, and taste function in anorexia nervosa.	30 hospitalized female patients. Age: 12 to 29 years.	Plasma, Hair and salivary Zn & Cu. Plasma iron, folic acid, iron binding, capacity, blood urea nitrogen & cholesterol.	RT and DT by Henkin's method TRS (taste recognition score) = 1 point for correct identification, max. score=20 points.	Plasma and urinary Zn were lower; Zn/Cu content of hair is normal. Hypogeusia to bitter and sour evident. Plasma Zn had no corr. with taste tests.
Cagan (1980)	Reply to letter by Henkin (1980) on zinc and taste.	Cagan invites interested readers to study the controversial literature on the therapeutic effectivness of zinc in treating taste disorders. Several studies are cited that support Henkin's view (Atkin-Thor et al., 1978), or refute his hypothesis (Burge et al., 1979).			
Cohen et al. (1973)	Thermal burn hypogeusia with altered zinc metabolism.	13 men + 60 women (9 to 70 years) with 2nd to 3rd degree burns. 62 men and 86 women as controls.	Zn/Cu in serum and urine. Increased Zn in urine noted.	RT & DT for 4 basic tastes (NACL,HCL, urea). 7 patients had ageusia, 5 patients had anorexia.	Decreased serum Zn but normal Cu in burn patients. Altered zinc metabolism is related to taste changes.
Friedman et al. (1980)	Zinc. supp. on taste acuity 0, 15, 50, 100 mg Zn.	Young women.	Plasma, whole blood, saliva hair, diet.	Triangle testing for basic tastes.	Zn supp. inc. Zn plasma and may improve sweetness perception.

Table 10, (continued)

Author	Type of Study	Subjects	Biological Measurements	Sensory Measurements	Conclusions
Greger (1977)	Dietary intake and nutritional status in the aged.	65 (31M, 34F), ave. age: 75.5 years.	10-day dietary intakes, ht., wt. Hair Zn. Meals tested for Zn and Mg. All intakes observed.	Taste acuity for salty and sweet (RT & DT): triangle tests, forced choice between and 3 conc. of NaCl, sucrose.	Dietary intakes were generally adequate except zinc and magn. 5% subjects had hair levels below 75ug/gm. 1/5 subjects had decreased taste acuity. Zn intake, hair Zn were not correlated to taste acuity.
Greger & Sciscoe (1977)	Zinc nutrition in the elderly & taste acuity.	44 subjects, mean age, 69 years (18M, 26F).	Questionnaire on food habits, 24-hr. dietary recalls, hair samples.	Taste acuity for DT for saltiness using triangle tests.	Subject had low intakes of zinc and low hair Zn levels. Taste acuity was less than that of young adults. 16% had very poor salt sensitivity.
Greger & Geissner (1978)	Zinc supp. in the elderly and taste acuity.	12 male and 13 female elderly (ave. age 76). Control group: 13 males, 11 females. (ave. age: 75).	Medical records, hair Zn, and 95 day menu evaluation for nutrient intake.	Taste acuity, RT & for salty and sweet triangle tests, forced choice between water and 3 conc. of NaCl and sucrose.	RT not affected by supp. for 95 days of 15mg Zn. Diets were low in hair Zn inc. after supp. Taste acuity not correlated to dietary factors, or hair Zn.

Table 10, (continued)

Author	Type of Study	Subjects	Biological Measurements	Sensory Measurements	Conclusions
Hambidge et al. (1972)	Anorexia, poor growth and hypogeusia in children.	338 children for hair Zn. 6 children for taste testing (ages 1 to 40 years).	Hair zinc. Used 1-2 mg $ZnSO_4$/Kg body weight.	3 drop method by Henkin. Appetite based on mother's evaluations.	Taste acuity low in 5/6 children. Zn normalized taste acuity and hair Zn levels increased. Zn def. may occur in "normal" children.
Hambidge et al. (1972b)	Taste acuity and hair zinc in normal children.	132 normal children (4-16 years). 84 subjects (3 months to 4 years for hair Zn analysis).	Hair Zn. 8/9 subjects had poor appetite and low height/ weight percentiles.	3 drop method. Taste acuity impaired in 5 subjects tested.	Decreased taste acuity repaired by 2mg/Kg/day of $ZnSO_4$ Mean hair Zn=157 ppm In 9 hypogeusiacs= 70 ppm.
Hambidge and Silverman (1973)	Zinc supplementation in infants with pica eating habits.	a 2 year old girl with a history of pica.	Hair zinc: 70 ppm. Given 10 mg $ZnSO_4$ once daily for 1 week, then 2 x daily with meat.	Poor appetite, and pica eating habits.	

Table 10, (continued)

Author	Type of Study	Subjects	Biological Measurements	Sensory Measurements	Conclusion
Henkin et al. (1963)	Taste thresholds in adrenal corifocal insufficiency (ACI).	9 (ACI) 9 normals.	Weight, serum NA and K.	Detection thresholds for MaCl, KCL $NaHCO_3$, sucrose urea, HCL. Used 3-drop technique.	Taste sensitivity in ACI 100 times the normal range. Prednisolone treatment reformed normal thresholds.
Henkin et al. (1967)	Taste change in D-penicillamine administration.	73 patients: with Scleroderma (12) cystinuria (12) arthritis (44) pulmonary fibrosis (5)	Ceruloplasmin, copper/levels in serum.	DT and RT of basic tastes by Henkin. Henkin concludes that copper and taste acuity related.	D-pen. reduced taste acuity in some (30%) patients but returned to normal when stopped. Copper. supp. returned taste function to normal.
Henkin (1968)	Olfaction/taste in pseudohypoparathyroidism.	6 patients aged 13-26 with PHP.	DHP patients confirmed by clinical measurements of serum Ca, P.	DT and RT for 4 basic tastes and vapors of pyridine, thiophene, nitrobenzene. 3-drop technique.	DT & RT higher than normal for sour, bitter. All vapors DT and RT higher than normal. Parathyroid extract or calcium supp. had no effect.
Henkin and Bradley (1969)	Taste acuity thiols and metal ions.	1 multiple myeloma patient.	Serum and urinary Zn/Cu.	Henkin's 3-drop method. For taste: RT and DT.	Patient had lowered DT & RT's. $CuSO_4$ returned taste acuity. $ZnCL^2$ also worked. Thiol drops lowered taste acuity.

Table 10, (continued)

Author	Type of Study	Subjects	Biological Measurements	Sensory Measurements	Conclusions
Henkin and Bradley (1970)	Hypogeusia and nickel (Ni) and zinc.	2 subjects: One with myelma and the other with cystinuria.	Serum and urinary Zn and Cu. Urinary Ni.	3-drop Henkin method. Added 60 mg Ni to diet and 60 mg Zn.	Hypogeusia can be corrected with Ni, Zn or Ca (lowered RT & DT's).
Henkin et al. (1971)	Idiopathic hypogeusia and taste and smell thresholds.	35 patients--(21 men, 14 women) (34-68 years) 21 controls.	Circumvallate papillae were examined by light and electron microscope.	DT & RT. Scaling on a 0-100 intensity scale of 4 basic tastes. DT & RT's for vapors of pyridine, nitrobenzene, thiophene.	Patients had elevated thresholds and decreased intensity scaling of 4 tastes No observable taste bud changes were noted in light microscope. Electron micr. noted vesiculation and vacuolization of taste papillae.
Henkin et al. (1972)	Taste and smell in Sjogren's Syndrome.	29 females with Sjogren's Syndrome. 10 subjects with parotid disease.	Biopsies of circumvallate papillae. Salivary flow rates, with depression of saliva subjects had taste loss.	Henkin's 3-drop DT & RT's, plus forced scaling. Usual 3 vapor DT and RT's.	Subjects lacked salivary flow. 52% had decreased appetites and hypogeusia. Most subjects had higher thresholds. Scaling did not reach 100% despite high concentrations. Lack of saliva means abnormal taste buds.

Table 10, (continued)

Author	Type of Study	Subjects	Biological Measurements	Sensory Measurements	Conclusion
Henkin et al. (1972)	Histidine and hypogeusia in normal subjects and patients with scleroderma.	5 patients with Sclero derma, 3 normals.	Serum and urinary Zn.	RT & DT's for 4 tastes (NaCl, sucrose, HCL and urea) and vapors (pyridine, nitro-benzene and thio-phene).	Histidine dosages (8-64g) increased taste and smell thresholds, and caused hypogeusia, anorexia and hyposma. 100 mg Zn restored acuities. Zn and histidine complex.
Henkin et al. (1975)	Acute Zinc Loss Syndrome.	Six patients (4F & 2M) with Systemic Sclerosis.	Serum and urinary Zn. 440 mg $ZnSO_4$ given orally.	3-drop Henkin method for taste and 3 bottle for smell.	Histidine intake caused anorexia, taste and smell dys-function-also noted: dec. in serum Zn and higher urinary Zn. Added Zn reversed this.
Henkin et al. (1975b)	Laser micro-probe analysis of the taste receptor. Zinc and taste acuity.	48 males, 55 females (25-81 years). All subjects had some viral infection or similar trauma. 42 patients treated with Zn. (25 & 100 mg).	Hemoglobin, hematocrit and RBC indices, WBC counts, serum K^+, Co_2, Ca, P, Mg, Zn, Cu, glucose, urea, creatinine, uric acid, liver enzymes, total protein, albumin cholesterol, etc. Parotid salivary flow rates.	3-drop Henkin method for taste acuity.	Single blind study of Zn. Zinc intakes de-creased all thresholds of taste in patients. Had poorer RT & DT's. Mean serum conc. for Zn were lower than controls, and Cu higher. Authors say zinc may control food intake.

Table 10, (continued)

Author	Type of Study	Subjects	Biological Measurements	Sensory Measurements	Conclusions
Henkin et al. (1976)	Randomized, double-blind crossover study of zinc on taste/ smell dysfunction.	106 patients with taste and smell dysfunction (mean age: 54.8).	Serum and urine Zn, Cu. Alkaline phosphatase in leukocytes and saliva collection.	3-drop DT & RT procedures of Henkin, forced scaling. 3 stimulus sniff technique of Henkin. Plus 0 to 100 scale for return of taste & smell to normal levels. Used 21 foods and 18 odors.	Mean serum Zn was low while Cu in normal range. Zn sulfate and placebo equivalent in inc. taste response. Mean parotid zinc levels below normal (16ppb). Inc. parotid zinc increases taste acuity. Serum or urine Zn not related to taste acuity.
Henkin (1980)	Letter to the Editor on zinc and taste.	Henkin reaffirms his stance on the effectiveness of Zn in taste restoration. Henkin indicates that serum, urine, hair, or other single tissue zinc levels are unreliable as mineral indicators. Only increases in salivary Zn correlate with taste improvement.			
Hussey (1974)	Editorial on Henkin's work and observations.	103 taste and smell dysfunction patients. 48 men and 55 women aged 25 to 81 years, ($\bar{X}$=55)	Serum Zn, urinary Zn.	Unknown, assumed Henkin's method.	96 patients had hypogeusia, with low serum Zn levels.
Rumble et al. (1975)	Zinc-65 in patients with taste and smell dysfunction.	20 patients taste/smell dysfunction.	Biological half-lifes of Zinc 65, given in 100mg/day dose.	Assumed Henkin's methods, but not specified nor were the results discussed.	Half time 297$\pm$33 days. For 13 of 20 subjects. 6 of 12 absorbed 65%, some less (28%) and more (99%)

Table 10, (continued)

Author	Type of Study	Subjects	Biological Measurements	Sensory Measurements	Conclusions
Schecter, Henkin, (1974)	Head trauma and taste/smell.	29 Patients after head trauma. 17 males, 12 females. (28-76 years) plus controls.	Serum and urine Zn/Cu low serum Zn. high serum Cu.	DT & RT. For NaCl, Sucrose, HCL and Urea. Vapors--pyridine, Nitrobenzene thiophene (DT & RT).	Typical symptoms include hypogeusia, dysgeusia, dysosmia. Most thresholds elevated in taste and smell.
Schechter and Prakash (1979)	Oral L-Histidine and zinc metabolism.	8 males (ages 32-38).	Body weights, serum and urinary Zn and histidine and food intake. Albumin bound Zn and macroglobulin bound Zn.	Used 10cm line scale for taste and smell perception and appetite.	Oral histidine, 4g/day, had no effect on appetite, sensory perception, food intake or body weight.
Smith et al. (1976)	Gustatory acuity in liver disease.	22 patients with acute viral hepatitis, 16 patients with chronic liver disease.	Liver function tests: bilirubin alkaline phosphatase, GOT, Vit.A. Serum Zn & Cu Retinol binding protein.	Taste thresholds and forced scaling (NaCL, Sucrose, HCL, urea).	Anorexia very common. Hypogeusia in 16 patients. As hepatitis improved, so did taste thresholds. Total serum Zn lower than normal. (32 vs. 92 mg/dl for chronic cirrhosis). Serum copper was elevated.

Table 10, (continued)

Author	Type of Study	Subjects	Biological Measurements	Sensory Measurements	Conclusions
Solomons et al. (1976)	Zinc nutrition in celiac sprue (CP).	10 females (28-79 years) with sprue; 10 females and 10 male controls. (19-43 years).	Serum Zn and Cu plasma albumin.	Modified 3-drop Henkin technique for 4 basic tastes. Plasma Zn and taste values did not correlate. Used taste detection score (TDS) method (-1) for each conc. missed.	CP had low serum Zn but normal Cu values. CP patients had more neg. TDS's. Zn def. common in adult celiac sprue.
		RECENT STUDIES			
Nishi et al. (1980)	Zinc status in chronic inflammatory bowel disease (CIBD).	30 CIBD; 17 normal children; 13 ch. of short stature, and 17 with anorexia nervosa (8-18 years).	Serum and urinary Zn. Zn tolerance test.	3-drop Henkin technique used.	All patients, even those with hypozincuria, had normal taste acuity.
Solomons et al. (1981)	Zinc Status & taste acuity in cystic fibrosis (CF).	19 juvenile CF, & 40 control adolescents.	Plasma and hair Zn, lung capacity.	Modified Henkin 3-drop technique.	CF had normal plasma Zn. CF had low hair Zn and higher taste thresholds.

Table 10. (Continued)

Table 10, (continued)

Author	Type of Study	Subjects	Biological Measurements	Sensory Measurements	Conclusions
Kosman and Henkin (1981)	Erythrocyte Zinc (EZ) in taste and smell dysfunction (TSD).	46 normals. 48 TSD's.	Serum erythrocyte Zn.	None	EZ poor indicator of exogenous Zn therapy--serum better.
Wright et al. (1981)	Zinc depletion and altered taste perception.	5 men in a metabolic ward.	Plasma and parotid Zn mean plasma Zn: 0.94ug/g.	Suprathreshold intensity scaling for NaCl and urea-saltiness and bitterness.	Zn depletion decreased saltiness perception, not bitterness. Plasma Zn, not parotid, decreased with depletion.
Freeland-Graves et al. (1981)	Taste acuity in vegetarians (VE) and non-vegetarians (NV).	100 VE and 100 NV.	Zn levels in plasma, saliva, hair and diet. VE plasma Zn: 103 vs. 121 mg/dl.	Triangle DT's for sucrose, NaCl, urea, citrate, MSG arginine, and chicken flavor. Odor recognition tests.	VE DT were lower on all tastes. Aroma identification for VE were also less in cottage cheese and beans.
Geliebter et al. (1981)	Oral L-histidine and taste acuity (letter).	3 obese females; 1 overweight male.	Serum and urinary Zn.	Methods based on Henkin's techniques.	No changes in taste and smell with L-histidine. 2 patients became anorectic.

Table 11. Reviews of Zn/Cu Metabolism and Sensory Evaluation

Author	Title of Paper	Material Covered	Comments
Catalanotto (1978)	Trace metal zinc and taste.	Brief review of selected papers on zinc and taste in humans and animals. Author raises criticism of previous human and animal work.	Author concludes that depletion of zinc can lead to decreased taste acuity but decreased taste acuity is not necessarily associated with depletion of zinc.
Nutrition Review (1979)	Ineffectiveness of zinc in treating ordinary taste and smell dysfunctions.	Careful analysis of Henkin's double-blind study, with mention of Greger's work on taste acuity in the aged.	Taste and smell disorders are of multiple etiologies. No scientific basis exists for using zinc in taste and smell disorders.
Anderson and Clydesdale (1978)	The many roles of copper in nutrition.	Briefly covers all aspects of copper nutrition including taste.	Good review of selected copper references. Includes discussion of Henkin's work.
Alter and Seltzer (1974)	What do taste and smell disturbances tell you?	Physiology and histology of patient care in taste/smell problems. Medical etiologies of sensory problems.	Mentions Henkin's data and the use of zinc sulfate in taste problems. Patient care oriented.
Henkins et al. (1969)	The molecular basis of taste and its disorders.	Reiteration of work by N.I.H. on taste, with rather bold hypothesis on taste mechanisms.	Fairly biased account of Henkin's work and what it may mean in taste perception.

REFERENCES

1. Atkin-Thor, E., Goddard, B.W., O'Nion, J., Stephen, R.L. and Kolff, W.J. Hypogeusia and zinc depletion in chronic dialysis patients. J. Am. Clin. Nutr., 31, 1948-1951, 1978.

2. Alter, M. and Seltzer, A.P. What do taste and smell disturbances tell you? Patient Care, Dec. 1, 76-88, 1974

3. Anderson, N.E. and Clydesdale, F.M. The many roles of copper in nutrition. Food Product Dev., 12, 39-40, 1978.

4. Anonymous. Ineffectiveness of zinc in treating ordinary taste and smell dysfuntion. Nutrition Reviews, 37, 283, 284, 1979.

5. Burch, R.E., Sackin, D.A., Jetton, M.M. and Sullivan, J.F. Decreased acuity of taste and smell in cirrhosis. Gastroenterology, 67, 781, 1974.

6. Burge, J.C., Park, H.S., Whitlock, C,P, and Schemmel, R.A. Taste acuity in patients undergoing long-term hemodialysis. Kidney International, 15, 48-53, 1979.

7. Cagan, R.H. Reply to Henkin. Nutrition Reviews, 38, 229-230, 1980.

8. Catalanotto, F.A. The trace metal zinc and taste. J. Am. Clin. Nutr., 31, 1098-1103, 1978.

9. Casper, R.C., Kirschner, B., Sandstead, H.H., Jacob, R.A. and Davis, J.M. An evaluation of trace metals, vitamins, and taste function of anorexia nervosa. J. Am. Clin. Nutr., 331, 1801-1808, 1980.

10. Cohen, I.K., Schechter, P.J. and Henkin, R.I. Hypogeusia, anorexia and altered zinc metabolism following thermal burn. J. AMA, 223, 914-916, 1973.

11. Freeland-Graves, J.H., Greninger, S.A., Young, R.K., Roby, M.J., Friedman, B.J. and Vann, K.L. Taste acuity in vegetarians and nonvegetarians. Fed. Proc., 40, 840, 1981.

12. Friedman, B.J., Freeland-Graves, J.H., Han, W.S., Young, R., Schlorlemer, M. and Geders, J. Effect of zinc supplementation on taste acuity in young women. Fed. Proc., 39, 651, 1980.

13. Geliebter, A.A., Hashim, S.A., and Van Itallie, T.B. Oral L-histidine fails to reduce taste and smell acuity but induces anorexia and urinary zinc excretion. J. Am. Clin.Nutr., 34, 119-120, 1981.

14. Greger, J.L. Dietary intake and nutritional status in regard to zinc of institutionalized aged. J. of Gerontology, 32, 549-553, 1977.

15. Greger, J.L. and Sciscoe, B.S. Zinc nutriture of elderly participants in an urban feeding program. J. Am. Diet.Assoc., 70, 37-41, 1977.

16. Hambidge, K.M., Hambidge, C., Jacobs, M. and Baum, J.D. Low levels of zinc in hair, anorexia, poor growth and hypogeusia in children. Pediat. Res., 6, 868-874, 1972a.

17. Hambidge, K.M., Hambidge, C. and Jacobs, M.A. Taste acuity, appetite, stature and hair zinc concentrations in "normal" children. Clin. Res., 20, 265, 1972b.

18. Hambidge, J.M. and Silverman, A. Pica with rapid improvement after dietary zinc supplementation. Arch. Dis. Childhood, 481, 567-568, 1973.

19. Henkin, R.I., Gill Jr., J.R. and Bartter, F.D. Studies on taste thresholds in normal man and in patients with adrenal cortical insufficiency: The role of adrenal cortical steriods and of serum sodium concentration. J. Clin. Invest., 42, 727-735, 1963.

20. Henkin, R.I., Keiser, H.R., Jaffe, J.A., Sternlieb, I. and Scheinberg, I.H. Decreased taste sensitivity after D-Penicillamine reversed by copper administration. Lancet, 2, 1268-1271, 1967.

21. Henkin, R.I. Impairment of olfaction and of the tastes of sour and bitter in Pseudohypoparathyroidism. J. Clin. Endocr., 28, 624-628, 1968.

22. Henkin, R.I., Graziadei, P.P.G. and Bradley, D.F. The molecular basis of taste and its disorders. Ann. Intern. Med., 71, 791-821, 1969.

23. Henkin, R.I. and Bradley, D.F., Regulation of Taste Acuity by Thiols and Metal Ions. Nat. Acad. Sci. Proc., 62, 30-37, 1969.

24. Henkin R.I. and Bradley, D.F. Hypogeusia corrected by Ni++ and Zn++. Life Sciences, 9, 701-709, 1970.

25. Henkin, R.I., Schechter, P.J., Hoye, R. and Mattern, C.F.T. Idiopathic hypogeusia with dysgeusia, hyposmia, and dysosmia. J. AMA, 217, 434-440, 1971.

26. Henkin, R.I., Keiser, H.R. and Bronzerti, D. Histidine-Dependent zinc loss, hypogeusia, anorexia, and hyposmia. J. Clin. Invest., 51, 44, 1972.

27. Henkin, R.I., Talal, N., Larson, A.L., and Mattern C.F.T. Abnormalities of taste and smell in Sjorgrens Syndrome. Ann. of Internal Medicine, 76, 375-383, 1972.

28. Henkin, R.I., Patten, B.M., Re, P., and Bronzert, D.A. A Syndrome of acute zinc losses. Arch. Neurol., 32, 745-751, 1975.

29. Henkin, R.I., Schecter, P.J., Raff, M.S., Bronzert, D.A. and Friedewald, W.T. Zinc and taste acuity: A clinical study including a laser microprobe analysis of the gustatory receptor area. In: "Clinical Applications of Zinc Metabolism," W. Pories, W. Strain, J.M. Hsu, and R.L. Woosley, Eds., Charles C. Thomas, Springfield, ILL. Ch. 19, pp. 204-228, 1975b.

30. Henkin, R.I., Schecter, P.J., Friedewald, W.T., Demets, D.L. and Raff, M. A double-blind study of the effects of zinc sulfate on taste and smell dysfunction. Am. J. Med. Sciences, 272, 285-299, 1976.

31. Henkin, R.I. Ineffectiveness of zinc in treating ordinary taste and smell dysfunction. Nutrition Reviews, 38, 228-229, 1980.

32. Hussey, H.A. Taste and smell deviations: Importance of zinc. J. AMA, 24, 1669-1670, 1974.

33. Kosman, D.J. and Henkin, R.I. Erythrocyte zinc in patients with taste and smell dysfunction. J. Am. Clin Nutr., 34, 118, 1981.

34. Nishi, Y., Lifshitz, F., Bayne, M.A., Daum, F., Silverberg, M., and Aiges, H. Zinc status and its relation to growth retardation in children with chronic inflammatory bowel disease. J. Am. Clin. Nutr., 33, 2613-2621, 1980.

35. Rumble, W., Aamodt, R., Johnston, G. and Henkin, R. Absorption and retention of zinc-65 in patients with taste and smell dysfunction. Clin. Res., 23, 393, 1975.

36. Schechter, P.J. and Henkin, R.I. Abnormalities of taste and smell after head trauma. J. Neurol. Neurosurg. Psychiatry, 37, 802-810, 1974.

37. Schechter, P.J. and Prakash, N.J. Failure of oral L-histidine to influence appetite or affect zinc metabolism in man: a double-blind study. J. Am. Clin. Nutr., 32, 1011-1014, 1979.

38. Smith, F.S., Henkin, R.I. and Dell, R.B. Disordered gustatory acuity in liver disease. Gastroenterology, 70, 568-571, 1976.

39. Solomons, N.W., Rosenberg, I.H. and Sandstead, H.H. Zinc nutrition in celiac sprue. J. Am. Clin. Nutr., 29, 321-325, 1976.

40. Solomons, N.W., Rieger, C.H., Jacob, R.A., Rothberg, R. and Sandstead, H.H. Zinc nutriture and taste acuity in patients with cystic fibrosis. Nutrition Research, 1, 13-24, 1981.

D.3. Use of Zinc/Copper to Restore Normal Taste/Odor Thresholds in the Elderly.

The literature reports two attempts to improve taste perception in the elderly. The first study involved the influence of improved oral hygiene, via a hygienist, on taste thresholds for the four basic tastes in twelve elderly institutionalized subjects (Langan and Yearick, 1976). The twelve selected subjects, members of a geriatric home and ranging in age from 52 to 86 years, underwent the oral prophylaxis for five weeks to remove gross calculus and debris from the teeth. Eleven control subjects received no prophylaxis but were visited three times a week by the dental hygienist. Detection and identification thresholds were tested by the dilution method of Dawson et al. (1963), in which subjects compared solutions of increasing concentration against distilled water.

Subjects varied widely in their ability to detect and identify the four primary tastes, with thresholds near those for younger adults. After oral hygiene treatments, the experimental group showed significant reductions in mean detection thresholds for sucrose and sodium chloride. The eleven control subjects were not affected. The authors concluded that taste perception, particularly for sweet and salty tastes, was enhanced by improving the health of the oral cavity. Although the experimental group increased their intakes of kcals, vitamins A, B_2 and B_1, calcium, protein and iron, a direct relationship between increased taste acuity and improved nutrient intake was not shown.

The second study by Greger and Geissler (1978) studied the effect of zinc supplementation on taste acuity in 49 institutionalized elderly. One group of 25 subjects received 15 mg of zinc sulfate daily for 95 days, while the other 24 subjects received a placebo daily. Taste acuity for salty and sweet, both detection and recognition thresholds, were measured by triangle tests of water versus three concentrations of each tastant. The detection thresholds for sodium chloride (NaCl) and sucrose improved slightly but not significantly, while recognition thresholds for NaCl and sucrose were unaffected. Taste acuity, according to the authors, was not correlated to dietary factors, hair zinc levels, smoking habits or use of dentures.

A recent study by Friedman et al. (1980) on zinc supplementation in young women attempted to determine if 15, 50 and 100 mg zinc dosages would influence taste acuity after 60 days. Taste acuity was measured via forced-choice triangle tests using the four basic flavor modalities of sweet, sour, salty and bitter. Although plasma zinc values increased in each supplementation group, salivary sediment and hair zinc did not significantly change within the four treatment groups. While a significant increase in taste acuity for sweetness existed in the 50 mg zinc group, the 100 mg group failed to exhibit improvement. The authors suggest that a transient rise in plasma zinc may improve the ability to taste sweetness.

REFERENCES

1. Dawson, E.H., Brudgon, J.L. and McManus, S. Sensory testing of differences in taste. I. Methods. Food Technology, 17, 1125-1131, 1963.

2. Greger, J.L. and Geissler, A.H. Effect of zinc supplementation on taste acuity of the aged. J. Am. Clin. Nutr., 31, 633-637, 1978.

3. Friedman, B.J., Freeland-Graves, J.H., Han, W.S., Young, R., Schorlemer, M. and Geders, J. Effect of zinc supplementation on taste acuity of young women. Federation Proceedings, 39, 651, 1980.

4. Langan, M.J. and Yearick, E.S. The effects of improved oral hygiene on taste perception and nutrition of the elderly. J. of Gerontology, 31, 413-418, 1976.

D.4. On Determining the Proper Levels of Zinc/Copper in Taste/Olfaction Restoration Studies.

In the development of the proper concentrations of both zinc and copper for supplementation purposes, several other biochemical considerations must be addressed:

1. Zinc inhibits copper absorption and vice-versa (Evans, 1973).
2. Klevay (1975) postulated a coronary heart disease link in diets with high zinc/copper ratios.
3. Zinc and copper sulfates may cause gastric irritation, nausea or malaise. Alternate conjugated forms of these mineral supplements may be more desirable (Oelshlegel and Brewer, 1977).
4. Estimated actual absorption of zinc and copper from dietary or supplemental sources (Mason, 1979).

Most authors would agree that zinc and copper have mutually antagonistic effects on absorption. Tsai and Lei (1979), in studying the consequences of cellulose on zinc/copper metabolism, noted a small but significant increase in the tibia zinc content in rats fed copper-deficient diets as compared to those fed the copper adequate diets. This observation was explained by a physiological competition for assimilation between the two metal ions. Hahn and Evans (1975) also noted that in their studies of metal homeostasis in zinc-deficient rats, copper may antagonize zinc metabolism by interacting with the metal-binding component in the intestinal fraction.

Taper et al. (1980) are of the opinion that the levels of zinc and copper normally found in the diet of man have little effect on the absorption of each other. They reiterate that dietary zinc only affects the utilization of copper when the Zn/Cu ratio is excessively high (500:1). Most human diets, they contend, possess zinc

to copper ratios in the range of 10:1 to 40:1. Copper retention, they caution, may be altered in those subjects being given therapeutic amounts of zinc for extended periods of time. The studies cited above provide supporting evidence that in zinc supplementation regimens, additional dietary copper may be necessary to prevent concomitant copper deficiencies.

Certainly to be controversial is the data presented by Klevay (1975) in support of his zinc/copper ratio hypothesis and coronary heart disease. Epidemiological and metabolic data, according to the author, are consonant with his hypothesis that a metabolic imbalance (high ratio) of zinc to copper in dietary regimens is a major factor in hypercholesterolemia and increased mortality. Although the author does not state what ratio of Zn/Cu may be suspect, the article leaves the impression that ratios greater than 10:1 are involved. While the hypothesis of an elevated Zn/Cu ratio in heart disease exposed by Klevay has not met with universal acceptance, the data and arguments presented merit some attention by researchers interested in zinc supplementation and sensory restoration. Consequently, the addition of a small amount of copper seems warranted in an effort to protect the best interests of all subjects involved in the studies.

Zinc sulfate, the most commonly used formula in human supplementation studies, is usually associated with minor gastric irritation in doses of 50 mg to 150 mg elemental zinc (Oelshlegel and Brewer, 1977). The authors contend that alternate forms of zinc ions, i.e., the citrate, gluconate or acetate forms, may be more tolerable in patients. Hence, it behooves the experimenter to provide a source of zinc and copper that minimizes the problems of gastric irritation

in experiments with voluntary compliance.

The final area of consideration in determining dosage levels is the percent absorption of the metal ions into the gastrointestinal tract--notably the small intestine (ileum). Sandstead (1973) surveyed the literature on this point and found, not surprisingly, that the dietary requirement of zinc is dependent upon its availability from food and its losses from the body. Reported estimates of the bio-availability from normal diets range from 1 to 58%, although most studies report 20 to 30% as the average value. In contrast, the National Academy of Sciences has set the dietary absorption of zinc at 40 percent in their determination of the recommended dietary allowances for zinc.

Despite the apparent discrepancies in the determination of zinc absorption, it remains important to recognize that oral supplements of zinc may not precipitate as great an increase in plasma, RBC or hair levels as the experimenter might be led to expect.

REFERENCES

1. Hahn, C.J. and Evans, G.W. Absorption of trace metals in the zinc-deficient rat. Am. J. Physiol., 228, 1020-1023, 1975.

2. Keppel, G. "Design and Analysis: A Researcher's Handbook." Prentice-Hall, Inc., Englewood Cliffs, N.J. 658 pp., 1973.

3. Klevay, L.M. Coronary heart disease: the zinc/copper hypothesis. J. Am. Clin. Nutr., 28, 764-774, 1975.

4. Sandstead, H.H. Zinc nutrition in the United States. J. Am. Clin. Nutr., 26, 1251-1260, 1973.

5. Taper, L.J., Hinners, M.L. and Ritchey, S.J. Effects of zinc intake on copper balance in adult females. J. Am. Clin. Nutr., 33, 1077-1082, 1980.

6. Tsai, R.C.Y. and Lei, K.Y. Dietary cellulose, zinc and copper: effects on tissue levels of trace minerals in the rat. J. Nutr., 109, 1117-1122, 1979.

III. METHODS AND PROCEDURES

A.1. Experiment I: Sensory Scaling, Salivary Flow Rates and Odor Recognition between Twenty-Five College Students and Twenty-Five Elderly Subjects.

College Subjects

Twenty-five Michigan State college students, both graduates and undergraduates, volunteered to be participants in the five week study, May 1, 1979 to June 9, 1979. Selection was based upon interest and availability for sensory testing. Table 20 gives the anthropometric particulars of the subjects (13 males, 12 females) who averaged 25.20 years, 174 cm in height, 65.5 kg in weight, and 16.6 in triceps fat-fold with a corresponding average of 25% body fat.

Elderly Subjects

The twenty-five elderly participants were chosen on the basis of availability and general health. Prior to selection, this author gave a short seminar to all the residents of Burcham Hills on September 5, 1979. The seminar outlined the purpose and objectives of the intended research. No attempt was made to conceal any information relating or pertaining to the intended research. Any biases that may be formed before the study by the elderly were considered in the design of the study.

A flyer was prepared and distributed to all the residents of Burcham Hills, only after permission was granted by Mr. Redheffer, Administrator in charge of operations. Figure I in the Appendix shows the verbatim letter put into all the mailboxes of the Burcham

Hills residents to encourage participation. In addition, an information bulletin was distributed that outlined the particulars of the study in question (Figure II, Appendix).

Initial response was disappointing; thus, a follow-up letter was distributed (Figure III, Appendix), encouraging the residents to witness an actual taste-testing session. With the help of Jane Greene, the activities director, and Mike Dauria, an expert in social communications, additional subjects were recruited.

The elderly participants also filled out a medical record form which inquired about drugs taken, vitamins used, and illnesses suffered by the participants prior to the initiation of the sensory studies (Table IV, Appendix). The intent of the study was to select a cross section of elderly subjects free from debilitating or life-threatening illnesses. According to Jane Greene, the subjects on our panel were the more enthusiastic and light-hearted members of the 240 residents at Burcham Hills. Jane mentioned to us that many of the other residents felt that their taste/smell acuity was not "good enough" for their participation or inclusion in the study. This feeling by some of the residents was later confirmed by personal observation, once Mike and I became friends with the 25 panel members. Thus, it would be important to reiterate that our taste panel did <u>not</u> represent a true cross section of elderly subjects, but reflected a healthier, more ebullient or exuberant subsection. Table XXI gives the anthropometric particulars of the elderly subjects (23 females, 2 males) who averaged 83 years, 158 cm in height, and 62 kg in weight.

Sensory Tasting

All college student taste testing was conducted in 301 Food Science Bldg. between the hours of one and four in the afternoon, three days a week, in the specially constructed taste booths which accommodated three subjects at a time. The sensory booths were constructed of particle board and painted with several coats of a latex, flat white paint. The booths were allowed to "season" for two months to insure that all the volatile components of the paint were removed.

The rationale behind using 301 Food Science instead of the regular taste booths for the college students was to simulate the conditions of the elderly taste testing at Burcham Hills to be completed at a later date. The booths and all the related taste/smell apparatus were easily constructed and movable for transportation purposes. Subjects were provided with taste treats at the completion of each day of sensory testing. A maximum of three subjects at a time were allowed, with scheduled times for each participant. Subjects who were sick or missed certain testing days completed their tests the following day or at a future time more convenient for them.

Elderly sensory testing was conducted from November 1, 1979 to January 12, 1980. Subjects were tested three times a week unless a holiday, such as Christmas, prevented the MWF taste testing. All sensory testing was conducted between one o'clock and four o'clock to assure the uniformity of sensory comparisons with the college subjects. The actual testing site was the "Snack Room ", a small, but very quiet room located in the basement, adjacent to the Beauty Shop. Tables and chairs were arranged such that four elderly subjects could be tested

simultaneously. Specially designed "Lazy Susan" booths isolated each participant during the sensory testing.

Consent Forms

Prior to the start of the taste studies, all panelists signed a consent form, prepared specifically for this study and approved by the All University Human Use Committee, as shown in the Appendix, Figure V.

PROCEDURES

Experiment I was divided into five sensory parts. The studies are summarized in Table 12.

A.2. Experiment I, Parts 1 and 2. Hedonic and Sensory Scaling of Sweetness Imparted by Sucrose in Lemonade.

Materials

1. Lemonade powder

Powdered lemon beverage base produced by General Foods Corp., White Plains, New York. Ingredients: citric acid, monocalcium phosphate, flavoring, F D & C color, Vitamin C, Vitamin A, and a clouding agent. Lemonade provided a convenient beverage in which to vary sweetness perception by addition of sucrose. Preliminary laboratory testing established five easily distinguishable concentration levels of sucrose to be used in hedonic and intensity testing (6, 8, 10, 14, 18% w/v).

2. Sucrose crystals

High purity, (analytical reagent) grade sucrose crystals manufactured by Mallinckrodt, Inc., Paris, Kentucky, 40361. Sugar was purchased in 5 lb. containers and used when needed. F.W.: 342.30. Chemical Formula - $C_{12}H_{22}O_{11}$.

3. Distilled water

All solutions were prepared with ion exchange distilled water using the Culligan purifying system present in the Food Science Building.

Sensory taste apparatus

Throughout the studies, 50 ml polypropylene beakers were employed which were coded with three digits using high visibility orange paint.

Preparation of Lemonade

Unsweetened lemonade base was prepared by dissolving 27 grams of powdered base into 3.8 liters of distilled water. Five preweighed amounts of sucrose corresponding to 6, 8, 10, 14, and 18% w/v sucrose were added.

METHODS

Hedonic Tasting

Judges rated degree of liking for sweetness in lemonade by circling a cross-bar on the 17-point, semi-structured hedonic scale labeled at each end with the terms "dislike extremely" and "like extremely" (Appendix, Figures VI-IX). Likewise, for sweetness intensity, subjects used the identical 17-point scale, but with the word anchors "extremely sweet" and "no sweetness". Between the tasting of all samples, distilled water for rinsing and a cuspidor for expectoration were provided.

Serving Conditions

All solutions were presented at room temperature (21^{o}C). The five samples were randomized and placed in aluminum serving pans prior to subject presentation. The use of randomized three-digit codes on

the polypropylene beakers and individualized taste booths prevented second guessing. In addition, all experiments had a trial practice run followed by three replications. If at any time the subjects had difficulty with the sensory testing procedures, the author explained the test over again, with additional trial runs if necessary. The elderly subjects had significantly more problems with the taste and smell sensory procedures. Thus, the experimenters took considerable time to explain all the testing procedures thoroughly.

Table 12. Chronological Summary of the Sensory Testing of College Students and Elderly In Experiment I.[1]

Study I.	Duration	Sensory Description
1.	2 weeks	Hedonic and intensity scaling of sucrose in lemonade. 3 Reps. 5 concentrations. (6, 8, 10, 14, 18% w/v).
2.	2 weeks	Hedonic and intensity scaling of salt in tomato juice. 3 Reps. 5 concentrations. (0, 1.0, 2.5, 4.5, 7.0% w/v).
3.	1 week	Odor identification of ten food odorants. 3 Reps.
4.	1 week	Intensity scaling of odor intensity. 3 Reps.
5.	1 week	Measurement of salivary flow rates using cotton dental rolls: testing, sniffing lemon wedges, tasting lemonade, and a final resting level. 3 Reps.

[1] See text for full description of sensory tests.

Experiment I. Part 2. Hedonic and Sensory Scaling of Saltiness Imparted by NaCl in Tomato Juice.

Materials

1. Sodium chloride (NaCl)

   One pound containers of Mallinckrodt, Inc., analytical grade sodium chloride were purchased. Formula weight: 58.44.

2. Tomato juice

   All tomato juice used was the unsalted brand "Featherweight" purchased through Central Food Stores on the Michigan State campus. Nutritional information, as stated on the label, is listed in Table X of the Appendix.

Preparation of Tomato Juice

Fifteen to twenty 18 fl. oz. cans of unsalted tomato juice were emptied into a large carboy for proper mixing. Five concentrations of NaCl in tomato juice were used--0.0, 1.0, 2.5, 4.5, and 7.0% w/v. All solutions were prepared at room temperature (21^{o}C) and stored in the refrigerator at 40^{o}F (4.5^{o}C) until needed. No opened, mixed tomato juice was stored for longer than three days.

Methods

Five samples of tomato juice with the five different levels of NaCl were served to each taste panelist in the same manner as that described for lemonade. Three replications of hedonic testing were followed by three replications of intensity testing for each subject.

The identical 17-point scale was used, with only a nomenclature change of the scoresheets from sweetness to saltiness. Tomato juice hedonic and intensity scoresheets are listed in the Appendix, Figures VI and VII.

Table 13. Identity of foods used as olfactory stimuli and their order of presentation in Experiment I.[1]

Replication 1	Replication 2	Replication 3
1. soy sauce	1. root beer	1. coffee
2. pepper	2. cocoa	2. soy sauce
3. grape	3. soy sauce	3. cocoa
4. cocoa	4. pepper	4. tea
5. tea	5. coffee	5. root beer
6. root beer	6. grape	6. grape
7. coffee	7. tea	7. pepper
8. cinnamon	8. orange	8. orange
9. orange	9. cinnamon	9. onion
10. onion	10. onion	10. cinnamon

[1]Only the first seven odorants were used for the intensity scaling work.

Table 14. List of food odorants presented to the elderly prior to third replication of odor recognition experiment. Experiment I, Part 3.[1]

Soy sauce*	Root beer*
cherry	molasses
pepper*	coffee*
lime	tuna fish
grape*	onion*
strawberry	orange*
cocoa*	cinnamon*
garlic powder	
tea*	

[1]*Asterisk indicates the actual ten food odorants.

A.3. Experiment I. Part 3. Odor Identification and Intensity of Ten Food Odorants.

Protocol Rationale

The purpose of part 3, Experiment I (odor identification) was to compare the olfactory responses to sniffing food odors without the use of associated sensory cues, i.e., vision, sound, etc. The criteria for selection of foods for odor identification included the purity of the volatiles produced and ready familiarity.

The final choices for food aromas and their order of presentation are listed in Table 13. As with the other parts of Experiment I, three replications were used, with care taken to keep weak odors preceding strong ones. The order of presentation of food aromas to the college students and the elderly residents was kept the same throughout the three replications. This was to assure that aroma order variance was identical in the two populations.

The college students were presented with the same odorants, in different orders, for three straight days. The elderly subjects were tested by the exact same sensory protocol, except, that on their third replicate, sixteen food odorants were listed on a sheet of paper and pasted on the booth wall for the elderly to view. The theory being that if memory loss was the primary problem resulting in poor recognition scores (see results section), then the "list" might measurably improve their performance (see Table 14).

Materials

The olfactory set-up enumerated below was performed the morning of the day's sensory testing to ensure fresh samples for the panelists.

Each of the following materials was placed in an individual 50 ml polypropylene beaker:

1. Cocoa (Nestle) powder--loosely fill the beaker to the 30 ml mark (19 g of cocoa powder).
2. Tea (Nestea)--place the instant tea into a beaker to the 20 ml mark and add water until the slurry levels off at the 20 ml mark.
3. Coffee (Folger's Perk)--fill the 50 ml beaker to the 30 ml mark (14 g).
4. Onion--mince a white onion and fill the beaker to the 30 ml mark.
5. Cinnamon (Krogers)--fill the powder to the 20 ml level (11.5 g).
6. Orange--cut up a fresh orange into small pieces and fill to the 30 ml level.
7. Pepper (Krogers)--pour 20 ml of the ground pepper into the beaker (12 g).
8. Soy Sauce (La Choy)--pour out 20 ml.
9. Grape (General Foods)--empty ½ of the grape "Kool-Aid" packet into the beaker and add water to the 20 ml mark and stir thoroughly.
10. Root beer (McCormick)--pour enough of the liquid essence of root beer to cover the bottom of the beaker (2.5 g).

After the preparation of the food materials, the appropriate amounts were added to 50 ml beakers covered by aluminum foil to eliminate visual inspection. Foil lids, that had 150 pinholes punched through them, were placed on the beakers as caps. Beaker lids were securely pressed into place by pressing with thumb around the rim. All samples were allowed to come to room temperature ($21^{o}C$). Four sets of the ten odorants were prepared in the morning and rotated immediately after use. A tray contained three to four odorants at a time. Each beaker was covered with a polypropylene cap to prevent aromas escaping from the hole-punched foil lids. These beakers were very effective in containing the food aromas. Subjects merely had to lift the cover, pick up the foil-lined 50 ml beaker and sniff the contents, then replace the cap. Three digit code numbers were used on the outside cap. Table XI in the Appendix contains additional information on the food items used. The polypropylene caps were kindly supplied by the L.J. Minor Corp. of Cleveland, Ohio 44113.

Preparation

All food items were assembled and prepared, as outlined above, on the morning of the testing day. The onions and oranges were purchased at a local grocery store and chopped approximately two hours before the actual olfactory testing.

Methods

Each taste panelist received a total of ten samples, with each tray holding three to four beakers. The first seven were to be scored

for odor recognition and intensity, while the last three were scored for odor recognition only. The scale used was a 200 mm horizontal line in which the panelists placed a vertical line corresponding to their perception of odor intensity. The line scale ranged from no odor to high odor intensity with reference foods given for each anchor point. Milk was chosen as an appropriate reference food stimulus for a low odor food, and vinegar was chosen as a high odor food reference. Care was taken to ensure that all panelists understood the placement of the vertical line scale and the direction of the testing methodology.

Subjects were required to sniff the reference foods to help anchor their judgments, then sniff the samples presented. First, subjects tried to identify the odor, guess if necessary, followed by the rating of the odor intensity. As will be described in greater detail in the results section, a number of the elderly failed to perceive the aroma of the vinegar reference (about 20%). In such cases, we asked the panelists to use their odor memories of strong food stimuli, such as vinegar, for the high odor intensity reference. An example of the odor recognition and intensity scoresheets are listed in the Appendix, Figure XII. Panelists were allowed to sniff repeatedly among the samples, if necessary.

Data Reduction

For odor intensity, each horizontal line placed by the residents onto the scoresheet was converted to a mm number starting from the left. The 200 mm line and scores taken from it were reduced by a factor of two, thus converting the line to 100 mm.

The odor recognition scores were essentially a three-point scoring system. Three points were awarded for a correct choice, two points for a guess that was regarded as "close" to the correct response, i.e., cherry for grape, and one point for a response such as "perhaps it is a fruit, but I am not sure". Zero points would be awarded for giving a response such as "tuna fish" for grape Kool-Aid. All panelists were instructed to describe the odorant if they could not name it.

In the odor recognition for the elderly, the third replication used a "list" of the odorants placed before them in the taste booth. Subjects merely sniffed the samples and selected the odorant from the given list. The choice of odorants on the list is given in Table 14. In such a system, the scoring was three points for a correct response (correctly identified the odorant) and zero points for <u>any</u> other response. The added discrimination of a full zero to three point scale was inappropriate when the list of the actual food odorants was given. It is also important to note here that the college students did not receive a list of the food odorants for their third replication.

A.4. Experiment I, Part 4. Salivary Secretion to Sniffing and Tasting Lemons and Lemonade.

Materials

Salivary Collection Equipment

1. Johnson and Johnson No. 2, 1-1½ inch cotton dental rolls.
2. 40 ml tared plastic vials with airtight lids.
3. Hever (W&R) stopwatch.
4. 7 inch stainless steel forceps.
5. General Foods lemonade with 10% w/v sucrose (mixed from powder).
6. Fresh lemons, chopped.
7. 70% isopropyl alcohol for disinfectant purposes.

Methods

Whole-mouth salivary secretion was collected using three pre-weighed cotton dental rolls. One roll was bent slightly to accommodate the narrow confinement in the mouth and was placed sublingually, and the two remaining rolls were placed buccally. All subjects completed the cotton roll placement within 15 seconds, insuring a uniform salivary baseline. After the one minute and 50 second stimulus presentation, the rolls were extracted within 10 seconds using stainless steel forceps. The rolls were replaced into the previously tared plastic vials and immediately weighed to the nearest mg.

When the rolls were in place, subjects were instructed to keep head and mouth movements to a minimum while carefully attending to

the stimulus. In addition, the head was kept in a horizontal plane to insure that the saliva was collected by the rolls and not pooled near the epiglottis, triggering the swallowing reflex. Each subject was tested four times. The first session was an orientation run designed to familiarize the panelists with the stimuli and collection procedures. The remaining three replications were used in the data analyses. As in all the taste sessions, food taste treats, such as cookies, were offered to interested taste panel members.

A sampling of 30 Johnson and Johnson No. 2 cotton dental rolls had a mean weight of 0.348 g, with a standard deviation of 0.0296 g. Total liquid absorptivity was 2.4 g distilled water per roll.

Stimuli Presentation:

1. 2 min. resting level (no stimulus present).
2. 2 min. sniffing of lemons (3 short sniffs every 15 sec., starting 15 sec. and ending 1:45).
3. 2 min. tasting of lemonade (3 drops on tongue every 30 secs., starting at 30 secs. and ending at 1:30).
4. 2 min. resting level.

One minute was allowed to elapse between stimuli presentations. As mentioned earlier, subjects put the three rolls into their own mouths, while the experimenter removed the rolls 1:50 min. later. The elderly had considerable difficulty with the placement of the cotton rolls in the proper positions, as if they had "forgotten" the exact location of the oral cavity. As a consequence, it took the elderly longer to place the rolls in the mouth, particularly under the tongue, than their student counterparts.

A.5. Experiment I. Part 5. Dietary Records.

In addition to the sensory studies outlined above, dietary records for three days were completed by both the elderly and college students. Data were collected for three days by the college students who recorded their own intakes, while the elderly intake during meals was recorded by Mike Dauria and this author.

The college students recorded their dietary intakes for three days- two weekdays and the following Saturday (during June 14-30, 1979), using the forms found in the Appendix, Table 13. Once the dietary records were completed, the foods were coded onto punchcards using the Michigan State University Nutrient Data Bank Code Book. The coding manual was developed under the joint efforts of Dr. Karen Morgan and Dr. Mary Zabik, members of the Dept. of Food Science and Human Nutrition. The data base itself was adapted from the HVH-CWRU nutrient data base, and is continually updated and revised as knowledge about nutrient content of food increases. If a food was not listed in the code book, then the closest food approximation was used in the analysis. If a food was a composite of several food groups and not listed in the code book, then every effort was made to find the appropriate recipe and code its food ingredients. The actual coding form used is listed in the Appendix, Table XIV. To illustrate its use, suppose a college student consumed 8 oz. of Total cereal, 1 teaspoon of non-dairy coffee whitener, 2 teaspoons of sugar, 2 cups of milk (whole), and 8 oz. of tea for breakfast. The decoder in this case would simply look up these food items in the code book, list the 6 digit code number under "item", code in the measure used (cups, oz., etc.) and fill in the

quantity, i.e., 2 cups, 2 oz. etc. For granulated white sugar, the item code is 700040, the measure allowed is a teaspoon, and the quantity used is 2.00 in this example.

The elderly subjects' dietary regimen was determined, for the most part, by the cyclic menu at Burcham Hills. Although the diets were somewhat restrictive, additional variance was allowable, as double entrees were a part of every meal. As the subjects sat down to their meal, we recorded all food items and intakes as closely as possible, including the post-meal weighing of unfinished foodstuffs. Portion sizes and weights were recorded prior to serving by actual measurement in the food service line. The cooperation of the head dietitian, Nancy Herbert, and food service personnel was greatly appreciated. The actual food service menu (Table XV) for the three day period is summarized in the Appendix.

Subjects merely selected the desired food groups off the listing, and after a short wait, the meal was served at their own table. Because Burcham Hills had two food serving shifts, the actual recording process was considerably less hectic. Those subjects who ate meals away from the facility or who consumed late night snacks conveyed this information to us for inclusion into their dietary records. Since portion sizes are critical to calculation of nutrient content of foods, the measuring scoop sizes used to serve the meals to the elderly were carefully studied to insure accurate serving sizes (see Figure XVI, Appendix).

A.6. Experiment I. Part 6. Hair Zinc/Copper Analysis.

In addition to the sensory studies and dietary recalls, hair samples were obtained (0.5-1.0 g) from each participant. Hair was snipped from the nape of the neck with stainless steel scissors. Care was taken to keep the hair samples of uniform length and as close to the scalp as possible. All samples were later wet ashed and analyzed for Zinc/Copper by atomic absorption spectrometry. See "Hair Washing and Ashing" under Experiment III, in the Methods section for more details on the exact procedure.

B.1. Experiment II. Second Olfactory Study with the Elderly and College Students.

The decreased olfactory acuity in the elderly during Experiment I, Parts 3 and 4, suggested a second experiment using the same elderly and college students who participated earlier. The experimental protocol of Experiment II focused upon the observed failure of the elderly to identify common grocery foodstuffs, such as pepper and root beer.

In Part A: Subjects were required to identify ten food odorants as before; however, two of the samples were actually "blanks", containing no odorous materials. A "list" of the odorants was not provided to the panelists in this first session.

In Part B: Subjects received the ten samples as in Part A, but a "list" was posted against the taste booth wall containing the eight food items plus six additional ones. In addition, all subjects were instructed that two of the samples were blanks.

A question was raised concerning the relative odor recognition abilities of the male vs. female college students participating throughout the sensory studies. In order to more closely study the potential difference, five more subjects were added to the 25 college participants, bringing the total male/female population to 15 each.

Materials

The olfactory set-up outlined below was prepared the morning of the taste session. All samples were purchased as fresh as possible, and disposed of after use. The following materials were placed into 50 ml polypropylene beakers:

1. Grape (General Foods)--empty 1/2 the packet into the beaker and add water to the 20 ml mark. Mix thoroughly.
2. Tuna Fish (Breast O'Chicken, Chunk Light)--lightly fill to the 20 ml mark.
3. Tomato Paste (Contadina)--fill to the 20 ml mark.
4. Lemon--dice a fresh lemon and fill to the 30 ml mark.
5. Pepper (Kroger Pure Ground)--pour 20 ml of the table pepper into the beaker.
6. Maple Syrup (Camp Pure Maple)--fill beaker to the 20 ml mark.
7. Apple Juice (Mott's Natural Style)--fill beaker to the 20 ml mark.
8. Garlic Powder (Kroger Pure)--fill beaker to the 20 ml mark.

Two blank beakers (which contained only water to make the weight similar to the samples) were also prepared, in addition to the eight food samples. Table XVII in the Appendix contains the nutrient and ingredient information of the aforementioned products. The order of the odorants for recognition sessions A and B are described in Table 15. The list given to the subjects during session B contained the eight food odorants plus six additional food items (see Table 15).

Procedure

The sensory procedures for Experiment II were very similar to those of Experiment I. The differences in food ingredients, and list vs. without list protocol, have been outlined previously. The use of blank samples in Experiment II should help discriminate those subjects who "just guess" and those more positive in aroma identification.

In addition, the use of blanks allows the experimenter some insight into which "classes" of food items the subjects might mistake for actual foods.

With the addition of the five college students, the age breakdown is as follows: 15 males, average age 25.3 ± 2.9; 15 females, average age 24.6 ± 4.3. To reiterate, all college students were either undergraduate or graduate students, with additional female subjects picked from the secretarial pool.

Table 15. Order of the food odorants presented to the elderly and college students in Experiment II.

First Session A	Second Session B
1. grape	garlic powder
2. tuna fish	-blank-
3. tomato paste	lemons
4. -blank-	tuna fish
5. lemons	apple juice
6. pepper	tomato paste
7. maple syrup	-blank-
8. -blank-	maple syrup
9. apple juice	pepper
10. garlic powder	grape

2) Actual list of the food odorants posted inside the taste booth in Session B, Experiment II.

tomato juice	pepper
apple juice	peaches
sardines	bananas
onions	maple syrup
grape	soy sauce
lemons	tuna fish
cranberry juice	garlic powder

Data Reduction

Scoring of the odor recognition results follows the identical guidelines of Experiment I, Part 3. Three points are awarded for a correct choice, two if the guess is reasonably close (such as cherry for strawberry), one if slightly related and zero if totally unrelated. In Part B, the panelists were presented with a list; therefore, the appropriate scoring was all or none. In other words, they either identified the food odorant correctly from the given list (three points) or failed to do so (zero points).

C.1. Experiment III. The Effect of Zinc/Copper Supplementation on Odor Identification, Intensity Scaling, and Salivary Flow Rates to Food Stimuli in Eight Elderly.

Subjects

Eight elderly subjects, previous participants in Experiments I and II, agreed to continue with the third phase of the experimental protocol--the supplementation study. Tables XVIII and XIX in the Appendix describe the handout given to all participants near the completion of Experiment II.

The actual supplementation period was from Feb. 1, 1980 to March 31, 1980. This two-month supplementation period was considered an adequate length of time to allow for assimilation and absorption. As a comparison, a general survey of the zinc/copper supplementation studies in the literature finds the average length of time for oral supplementation to be approximately two months (Table 16).

Experimental Protocol Materials

Randall Health Foods--zinc citrate (50 mg elemental zinc) and amino acid chelated copper (5 mg elemental copper)--were supplied to each elderly participant in the study. A sixty-day supply of each tablet was provided, along with the instructions to take the copper tablet in the morning after breakfast and the zinc tablet after dinner. Subjects who experienced memory lapses and failed to take their tablets were not requested to "catch up" by taking two tablets the next day.

Table 16. Concentrations of Zinc/Copper and length of the supplementation period in studies using metal ions to restore sensory acuity.

Author	Research Area	Supplementation Amounts and Length
Hambidge et al. (1972	Taste acuity in children	$ZnSO_4$ 2 mg/kg/day for 1-3 months
Friedman et al. (1980)	Taste acuity in young women	15, 50, 100 mg zinc for 60 days
Atkin-Thor et al. (1978)	Hypogeusia in dialysis patients	220 mg $ZnSO_4$ for 6 weeks. (85 mg Zn)
Henkin et al. (1976)	Double-blind zinc supp. study	100 mg zinc as $ZnSO_4$ for 1-1/2 months
Greger and Geissler (1978)	Zinc supp. and taste in the elderly	15 mg zinc as $ZnSO_4$ for 95 days
Henkin et al. (1967)	Taste sensitivity and copper administration	5-15 mg copper as $CuSO_4$ for 4 weeks

Sensory Evaluation

At the conclusion of the two month period, the elderly were retested on the identical sensory protocols of odor recognition, intensity and salivation, as described previously in Experiment I. Since the sensory testing of gustation in the elderly of Experiment I found little evidence of decreased taste acuity, the eight elderly subjects were not retested in this area. Furthermore, some subjects experienced fatigue along the course of the sensory retesting; the decision to eliminate the gustatory protocol was made.

Hair Analysis

At the beginning of the sensory testing and at the end of the supplementation period of Experiment III, hair samples were obtained (0.5-1.0 g) from participants. Hair was snipped from the nape of the neck with stainless steel scissors. Care was taken to keep the hair samples of uniform length and as close to the scalp as possible. All samples were later wet ashed and analyzed for zinc/copper by atomic absorption spectrometry, as described in a following section on hair washing and ashing.

METHODS

Olfactory Testing

At the end of the supplementation period, subjects were retested on olfactory recognition and intensity to the identical foods used in Experiment I, Parts 3 and 4. Subjects were required to first identify the odorant and then rate the intensity. The order of the testing was:

1. Odor identification of the ten food odorants without a list, then an intensity rating of seven food odorants (1 rep.).
2. Odor identification of the ten food odorants with a list, followed by an intensity rating of seven food odorants (1 rep.).
3. Final intensity scaling of seven food odorants (1 rep.).

Salivary Testing

Salivary flow rates were measured in the eight subjects, as described in Study I. To reiterate, 2-min. salivary flow rates were measured in response to:

1. 2 min. resting level (no stimulus present)
2. 2 min. sniffing of lemons
3. 2 min. tasting of lemonade
4. 2 min. resting level (no stimulus present)

Finally, at the end of the experiment, all subjects were reweighed and asked for any subjective feelings on whether the mineral therapy was affecting their sensory preparation. A list of their comments may be found in the Appendix, Table XXV.

Hair Washing and Ashing

Hair Washing

All collected hair samples were subjected to the following cleansing procedure prior to the wet-ashing. The procedure used was a modified version of the methodology by Klevay (1970). Hair samples were collected and finely chopped with stainless steel clippers. After chopping, the samples were placed in individual plastic vials

and washed according to the following:

1) acetone--3 times
2) 1% (Triton X)--3 times
3) Distilled water--3 times

The washing procedure consisted of shaking the sample in the selected washing medium for 2 minutes, whereupon the solution was drained off. Individual 50 ml polypropylene beakers were used throughout the analysis to avoid metal ion contamination. After the final rinsing with distilled water, the samples were allowed to dry overnight in a calcium chloride dessicator.

Wet Ashing

The dried hair samples were weighed accurately to three places on an analytical balance. Care was taken in the weighing and transfer to insure against loss of small hair fibers in handling. After transfer to glass ashing vials, 3 ml of concentrated nitric acid was added to each of the tubes. Distilled water was added as a washdown along the sides of the boiling flask. Samples were then heated to boiling for approximately 30 minutes or until a small amount of clear, nonfuming liquid remained. Digested samples were quantitatively transferred to clean polypropylene beakers and brought up to 10 ml final dilution with distilled water.

After the appropriate standard solutions for zinc and copper were prepared, samples were analyzed using a Hitachi 180-70 Model Atomic Absorption Spectrophotometer (AAS). The cooperation of the Carnation Company for the assistance and use of their AAS is greatly appreciated.

REFERENCES

Klevay, L.M. Hair as a biopsy material. Assessment of zinc nutriture. J. Am. Clin Nutr., 23, 284-289, 1970.

IV. RESULTS

A.1. Experiment I. Sensory Scaling, Salivary Flow Rates and Odor Recognition between Twenty-Five College Students and Twenty-Five Elderly Subjects.

Parts 1 and 2: Hedonic and Intensity Scaling of Sucrose in Lemonade and Tomato Juice.

Subjects

Twenty-five college-age subjects and 25 elderly subjects volunteered to participate in Experiment I. Table XX, in the Appendix, details the anthropometric data of the college participants, who averaged 25.2 years of age, 65.5 kg in weight and 25.0% body fat. The college group consisted of 12 females and 13 males, picked from a pool of graduate students and secretaries working in the Food Science Building.

Table XXA, of the Appendix, describes the anthropometric particulars of the 25 elderly residents of Burcham Hills. Subjects averaged 83.5 years in age and 62.0 kg in weight. The group, whose age ranged from 74 to 95 years, consisted of 23 female and 2 male subjects. Appendix Table XXII summarizes the anthropometric data on the two subject groups for side-by-side comparison.

The elderly subjects were required to fill out a brief medical survey that asked their history of drug and vitamin ingestion, smoking and current illnesses. Appendix Table XXIII summarizes the questionnaire and the findings. A majority of the subjects questioned were taking medication (76%) at the time, but only 32% were concurrently taking vitamins. Two of the subjects were diabetic, one had kidney stones, and eight were hypertensive. None of the subjects were

smokers. Table XXIV, in the Appendix, lists the drugs mentioned in the medical record of the elderly.

1. Hedonic Response to Tomato Juice

Figure 1 illustrates the average hedonic and intensity response to tomato juice by the 25 elderly and college subjects. Inspection of the hedonic responses, by both groups, reveals a similar 1.0% NaCl-peaking sensory function. Because of the great variability in individual responses, the slight elevated function for the elderly would usually have little significance.

However, there was a highly significant interaction by Group and Concentration (A X C) (see Table 17). This significance is suggested by visual inspection of the consistently elevated elderly response to the degree of liking of the various salt levels. Of considerable interest is the non-significant judge F-ratio (1.09) indicating good subject agreement for all concentrations. The low F-ratio for replications again highlights the ability of the judges to replicate their own sensory evaluations.

2. Intensity Response to Tomato Juice

A similar situation exists in the intensity ratings of the tomato juice by the two groups. Figure 1 depicts a narrower intensity range of response by the elderly (5 to 15.5 points) than the college students (1.5 to 16 points).

Table (18) shows a significant F-ratio for groups (elderly vs. college) in the grand mean, individual judges, concentrations, but not in replications. The most important interaction (A X C), groups

X concentration, was highly significant, indicating a difference of opinion in the intensity judgements by college students and the elderly.

Figure 1. Comparison of mean hedonic and intensity responses to varying NaCl concentrations in tomato juice, Experiment I.1.

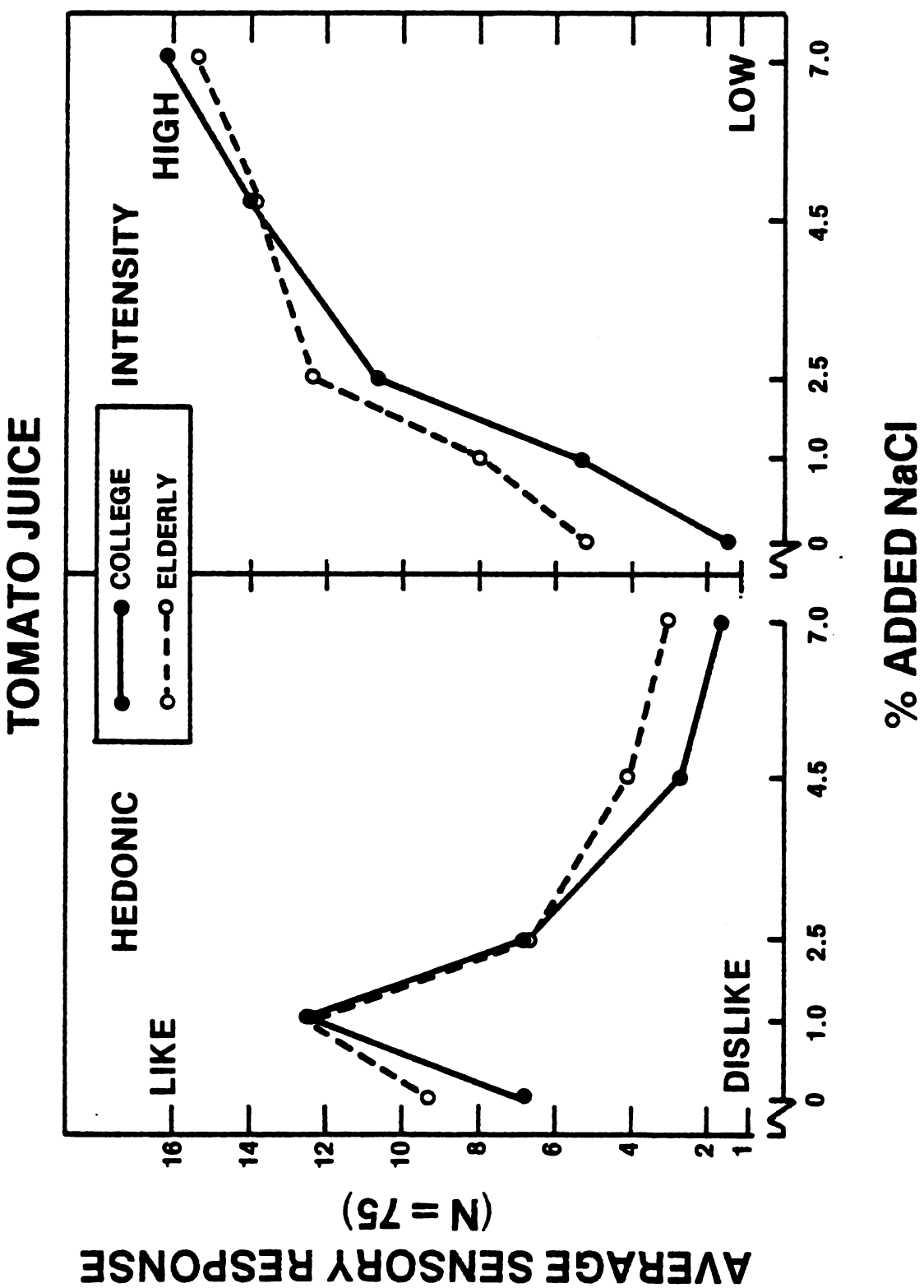

TOMATO JUICE
HEDONIC
INTENSITY
LIKE
DISLIKE
HIGH
LOW
COLLEGE
ELDERLY
AVERAGE SENSORY RESPONSE
(N = 75)
% ADDED NaCl
16
14
12
10
8
6
4
2
1
0
1.0
2.5
4.5
7.0

Table (17). Nested analysis of variance: Degrees of freedom (D.F.), the sum of squares (S.S.) and F-ratios for the hedonic responses to saltiness in tomato juice by the elderly and college students; in Experiment I.1.

HEDONICS--TOMATO JUICE

Source of Variation	D.F.	S.S.	M.S.	F-Ratio	Test Source[1]
A-Groups	1	213.34	213.34	12.76***	B
B-Judges	48	802.78	16.72	1.09	Error
C-Concentrations	4	9588.54	2397.14	221.55***	BC
D-Replications	2	10.72	5.36	0.92	BC
A X C	4	197.34	49.34	4.56**	BC
B X C	192	2077.86	10.82	0.70	Error
A X D	2	1.7	0.85	0.80	BC
B X D	96	557.22	5.80	0.38	Error
C X D	8	60.94	7.62	0.50	Error
A X C X D	8	94.46	11.81	0.77	Error
ERROR	384	5906.62	15.38		
TOTAL	749				

[1]The Source of the denominator used to calculate the tabulated F-ratio.

, *, Significant at P<0.01, and 0.001, respectively.

Table (18). Nested analysis of variance: degrees of freedom (D.F.), sum of squares (S.S.) and F-ratios for the intensity responses to tomato juice by the elderly and college students; in Experiment I.1.

	INTENSITY--TOMATO JUICE				
Source of Variation	D.F.	S.S.	M.S.	F-ratio	Test Source[1]
A-Groups	1	384.5	584.5	18.67***	B
B-Judges	48	984	20.50	1.72*	Error
C-Concentrations	4	15963.4	3990.85	570.12***	BC
D-Replications	2	18.73	9.37	2.18	BD
A X C	4	528.06	132.02	18.86***	BC
B X C	192	1344.86	7.0	0.59	Error
A X D	2	13.03	6.52	0.93	BC
B X D	96	411.53	4.29	0.36	Error
C X D	8	53.89	6.74	0.57	Error
A X C X D	8	18.02	2.25	0.19	Error
ERROR	384	4575.74	11.92		
TOTAL	749				

[1]The source of the denominator used to calculate the tabulated F-ratio.

*, ***, Significant at P<0.05 and 0.001, respectively.

3. Hedonic Responses to Lemonade

Table (19) summarizes the results of the nested analysis of variance for the hedonic responses of the elderly and college students to varying levels of scurose in lemonade (Figure 2). The hedonic scaling by the elderly followed closely to that of the college students for the first three concentration levels of sucrose (6, 8, 10% w/v), then increased slightly in the last two concentrations (14, 18% w/v). This difference was significant, but barely so (5% level). Although judges did not differ significantly from each other, the replications per judge did.

4. Intensity Responses to Lemonade

Inspection of the intensity graphics to sweetness in lemonade (Figure 2) demonstrates a greater disparity in response by the two groups than to the NaCl in tomato juice. As with the NaCl graphics in tomato juice, the elderly displayed a constriction in sweetness intensity range (6.3 to 14.6%) compared to the college students (2.7 - 15.8%). Both the group and the group X concentration interaction was significantly different, as shown in Table (20). Although the replicate samples differ from each other (F-ratio: 4.58*), the magnitude of the group by concentration interaction was so large (F-ratio: 26.23) that it overshadowed the increased sample variability.

Table (21) contains the hedonic and intensity means for groups (elderly vs. college) and tastant concentrations for Experiment I, Part 1.

Table (19). Nested Anova (elderly vs. college students) for hedonic responses to sucrose in lemonade.

Source of Variation	D.F.	S.S.	M.S.	F-ratio	Test Source[1]
A-Groups	1	173.77	173.77	5.98*	B
B-Judges	48	1395.19	29.07	1.45	Error
C-Concentrations	4	2033.02	508.26	44.23***	BC
D-Replications	2	34.33	17.17	3.41*	BD
A X C	4	128.78	32.18	2.80**	BC
B X C	192	2206.33	11.49	0.57	Error
A X D	2	15.22	7.61	0.66	BC
B X D	96	482.75	5.03	0.25	Error
C X D	8	53.64	6.71	0.33	Error
A X C X D	8	67.73	8.47	0.42	Error
ERROR	384	7705.65	20.07		
TOTAL	748				

[1] The Source of the denominator used to calculate the tabulated F-ratio.

*,**,***, Significant at P<0.05, 0.01 and 0.001, respectively.

Figure 2. Comparison of the average hedonic and intensity responses to varying sucrose levels in lemonade, Experiment I.2.

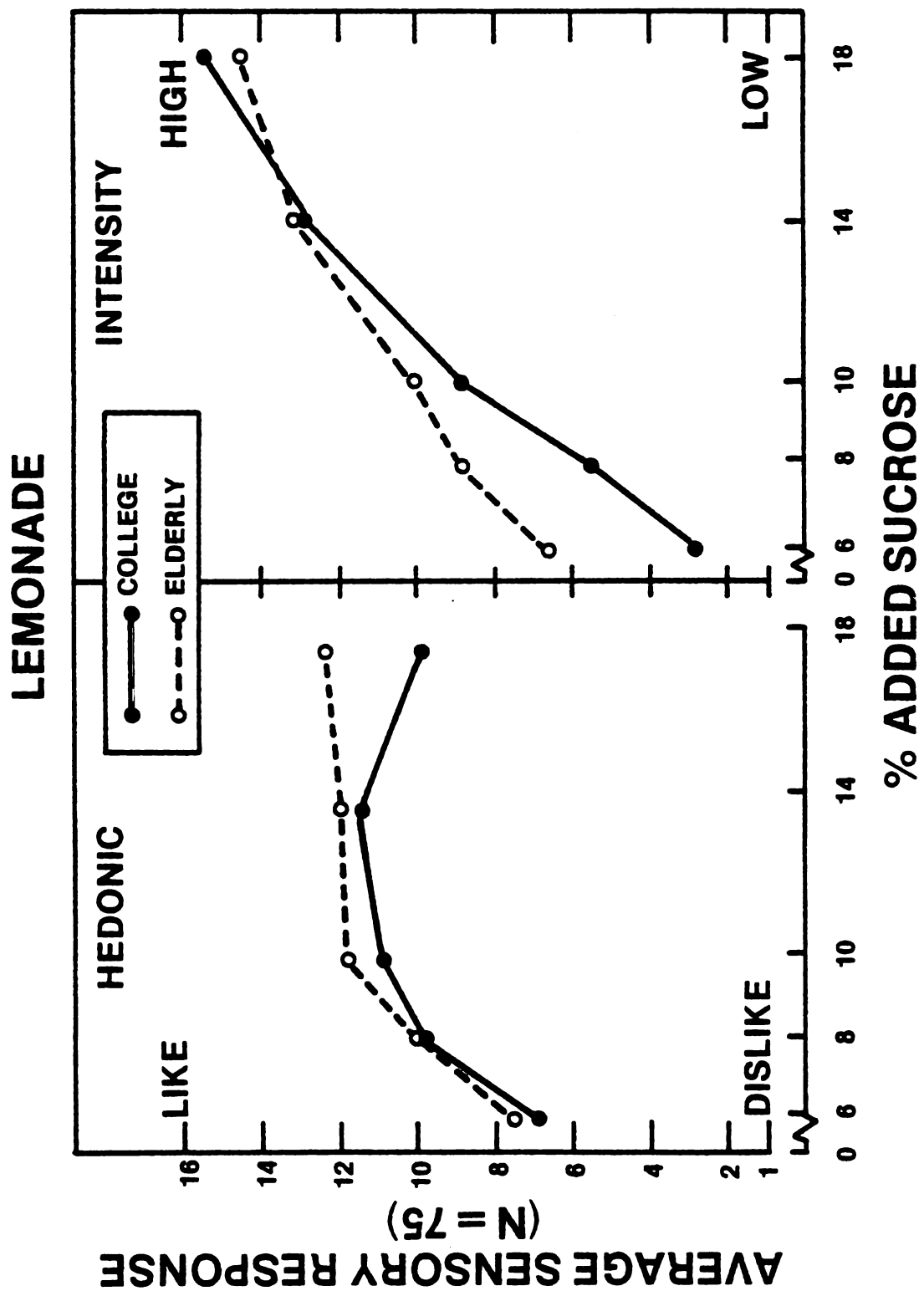

LEMONADE
HEDONIC
INTENSITY
LIKE
DISLIKE
HIGH
LOW
COLLEGE
ELDERLY
AVERAGE SENSORY RESPONSE
(N = 75)
% ADDED SUCROSE
16
14
12
10
8
6
4
2
1
0 6
8
10
14
18 0 6
8
10
14
18

Table (20). Nested Anova (elderly vs. college) for intensity responses to sucrose in lemonade.

		INTENSITY--LEMONADE			
Source of Variation	D.F.	S.S.	M.S.	F-ratio	Test Source[1]
A-Groups	1	428.67	428.67	19.78***	B
B-Judges	48	1041.34	21.67	1.74*	Error
C-Concentrations	4	10249.8	2562.45	494.68***	BC
D-Replications	2	42.58	21.29	4.58*	BD
A X C	4	543.39	135.85	26.23***	BC
B X C	192	994.72	5.18	0.42	Error
A X D	2	46.94	23.47	4.53*	BC
B X D	96	446.75	4.65	0.37	Error
C X D	8	43.48	5.44	0.44	Error
A X C X D	8	28.73	3.59	0.29	Error
ERROR	384	4768.97	12.42		
TOTAL	749				

[1]The Source of the denominator used to calculate the tabulated F-ratio.

*, ***, Significant at P<0.05 and 0.001, respectively.

Table (21). Hedonic and intensity means for groups (college vs. elderly), and concentrations (of NaCl) for the tomato juice and lemonade food systems of Experiment I.

TOMATO HEDONICS					LEMONADE HEDONICS				
A-Groups:***					A-Groups:*				
Elderly				College	Elderly				College
7.11				6.05	10.78				9.81
C-Concentrations:***					C-Concentrations:***				
0.0	1.0	2.5	4.5	7.0	6	8	10	14	18
8.06	12.42	6.62	3.42	3.04	7.24	9.90	11.38	11.76	11.18

TOMATO INTENSITY					LEMONADE INTENSITY				
A-Groups:***					A-Groups:***				
Elderly				College	Elderly				College
10.99				9.56	10.63				9.11
C-Concentrations:***					C-Concentrations:***				
0.0	1.0	2.5	4.5	7.0	6	8	10	14	18
3.38	6.71	11.48	14.0	15.58	4.76	7.22	9.46	12.94	14.98

[1]Underlined means within a row do not differ significantly.

*, ***, Significant at $P<0.05$ and 0.001, respectively.

Of interest is the consistently higher group means for the elderly throughout the two food systems. The subjects, both elderly and college, had an easier time separating the five concentrations of lemonade by intensity, as evidenced by the greater number of mean separations, than the NaCl in tomato juice. Because the hedonic function for sweetness in lemonade is an inverted "U" shape, levels 14-18% sucrose were not separated well by the elderly panelists.

Tables (22) and (23) contain the separate analysis of variance for the elderly and college students in both the hedonic and intensity scalings of tomato juice and lemonade. In the replications of their individual sensory scalings, only the elderly (intensity, lemonade) had any difficulty. However, in the F-ratios for the other three food systems, the elderly matched the college subjects' low variabilities. As far as the concentration and subjects' variations are concerned, both groups displayed remarkable similarity.

Figure 3 depicts the average sensory response to tomato hedonics and intensity in five classes of elderly response types. For example, subject number four, in the upper left, displays the "ideal" opposite bifurcation of the sensory functions of hedonics and intensity. In contrast, subject number 13 demonstrates a "flat" intensity curve, suggesting a decreased ability to taste NaCl in tomato juice. Five elderly subjects, in total, displayed a flat intensity curve, as seen by subject number 13. However, the same subjects did rate the hedonics of the higher concentrations in a downward fashion for all four of the "flat" intensity curves.

Table (22). Separate AOV for the hedonic response to sucrose in lemonade and NaCl in tomato juice, Experiment I.

Source of Variation	D.F.[1]	Tomato Juice		Lemonade	
		Elderly F	College F	Elderly F	College F
A-Concentration	4	123.281***	259.831***	30.076***	36.634***
B-Subjects	24	4.440***	5.347***	4.804***	10.047***
C-Replication	2	0.344	0.987	1.884**	0.355
A X B	96	2.440**	3.793***	2.696**	3.982***
A X C	8	1.407	1.207	1.212	0.696
B X C	48	1.494	1.548*	1.640*	1.299***
A X B X C	192				
TOTAL	374				

*, **, ***, Significant at P<0.05, 0.01, and 0.001 respectively.

[1]D.F. is degrees of freedom and F is the F-ratio.

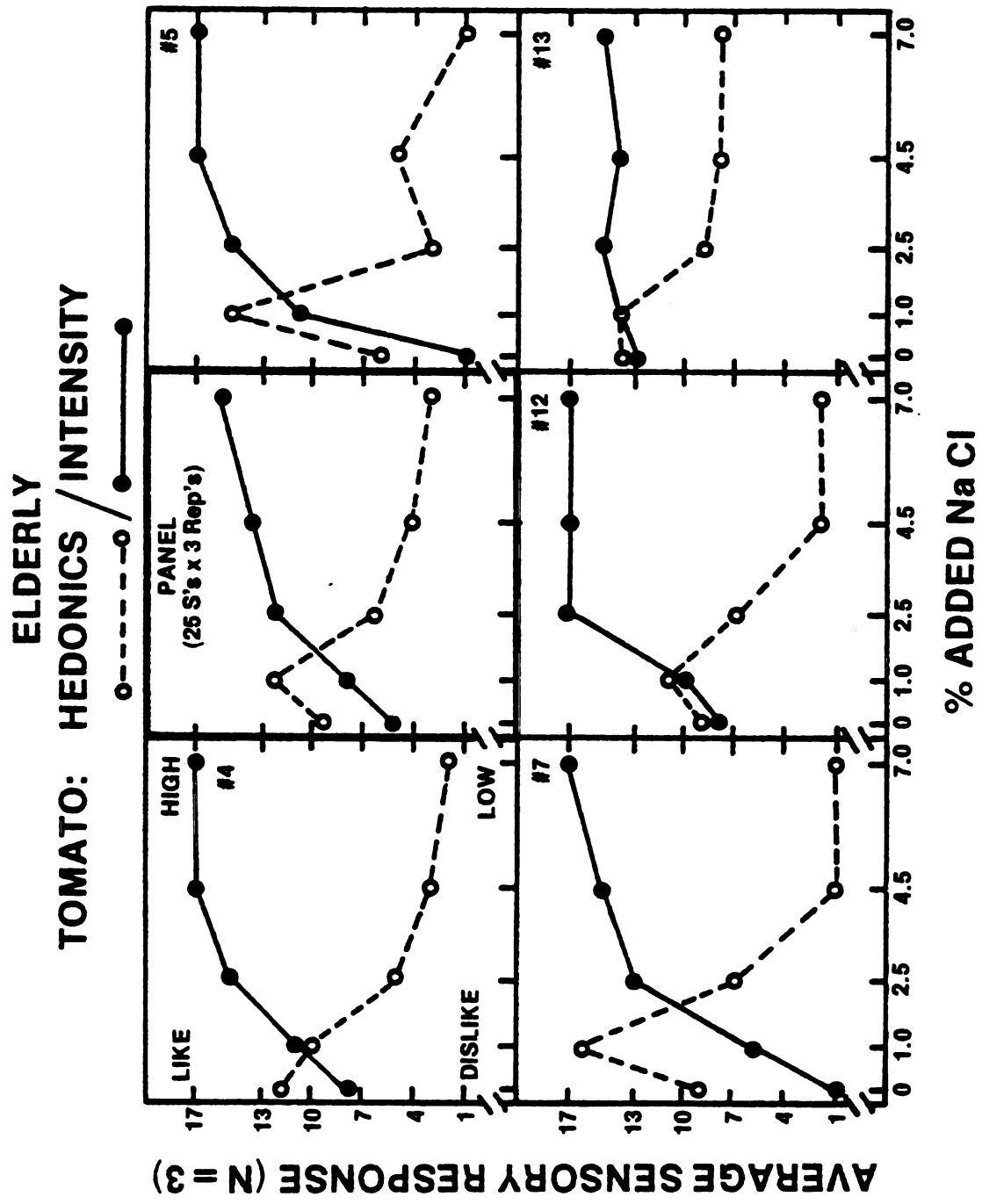
Table (23). Separate AOV for the intensity responses to sucrose in lemonade and NaCl in tomato juice, Experiment I.

Source of Variation	D.F[1]	Tomato Juice		Lemonade	
		Elderly	College	Elderly	College
A-Concentration	4	144.132***	1279.34***	94.689***	842.229***
B-Subjects	24	5.709***	8.912***	8.250***	11.875***
C-Replication	2	1.663	0.302	5.506**	0.0856
A X B	96	2.206**	2.954***	1.736**	3.184***
A X C	8	0.724	1.037	1.021	0.332
B X C	48	1.273	1.822**	1.733*	2.528***
A X B X C	192				
TOTAL	374				

*, **, ***, Significant at P<0.05, 0.01, and 0.001 respectively.

[1]D.F. is degrees of freedom and F is the F-ratio.

Figure 3. Individual sensory graphs of five sensory types to tomato intensity and hedonics by the elderly in Experiment I.1.


ELDERLY
TOMATO: HEDONICS / INTENSITY
PANEL
(25 S's x 3 Rep's)
#4
#5
#12
#13
#7
HIGH
LOW
LIKE
DISLIKE
AVERAGE SENSORY RESPONSE (N = 3)
% ADDED Na Cl

This would suggest that the elderly subjects, although unable to differentiate the higher levels of NaCl, were still able to rate the solutions as unpleasant.

Figure 4 illustrates five examples of individual sensory scaling by the college students to tomato intensity and hedonics. Note the distinctive sensory response profiles of hedonic versus intensity scaling. None of the college students had any difficulty separating out the higher levels of NaCl concentrations.

Figures 5 and 6 depict the individual sensory graphs of five response types to lemonade intensity and hedonics by the elderly and college students, respectively. In the lemonade food system, ten elderly subjects exhibited "flat" intensity functions as the concentration of sucrose increased. In Figure 5, subject number 18 is an example of the "flat" response. A confused response, one in which a concentration is not scaled correctly, is shown by subject number 25, in the lower right corner of Figure 5. Ten elderly subjects had some difficulty correctly scaling the sucrose concentrations in order, although this confusion is not obvious by inspection of panel average.

Table (24) summarizes the number of elderly subjects with "flat" or "confused" sensory scaling functions. A question might be asked concerning those subjects who fall into the flat or confused response category: Do they repeat themselves in both taste systems? By observing Table (24), one finds that three elderly with flat functions for tomato juice were also flat in lemonade. In addition, six individuals were possessors of flat and confused sensory functions in the tomato juice and lemonade.

Figure 4. Individual sensory graphs of five response types to tomato intensity and hedonics by the college students in Experiment I.1.

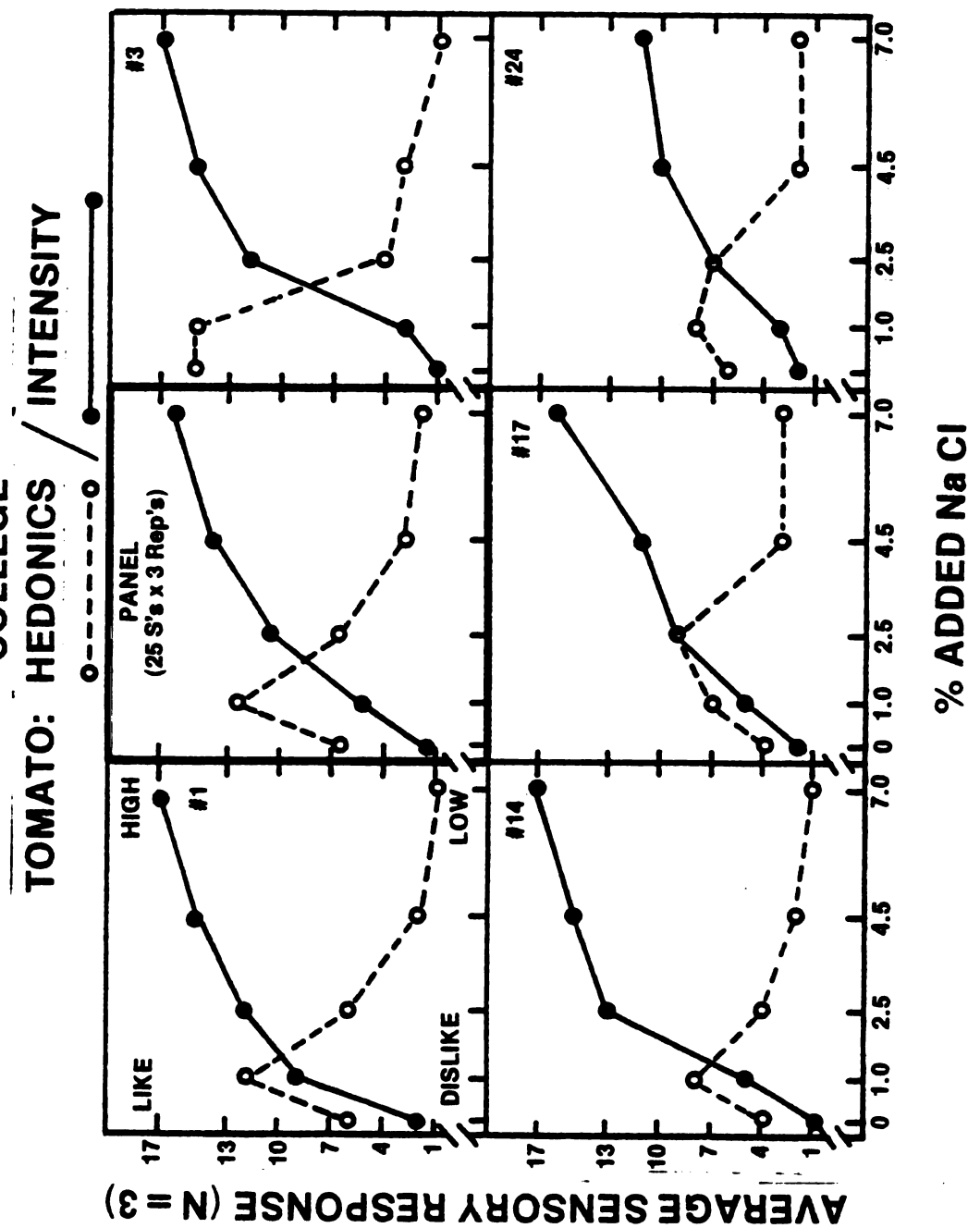

COLLEGE
TOMATO: HEDONICS / INTENSITY
PANEL
(25 S's x 3 Rep's)
HIGH
LOW
LIKE
DISLIKE
#1
#3
#14
#17
#24
AVERAGE SENSORY RESPONSE (N = 3)
% ADDED Na Cl

Figure 5. Individual sensory graphs of five response types to lemonade intensity and hedonics by the elderly subjects in Experiment I.2.

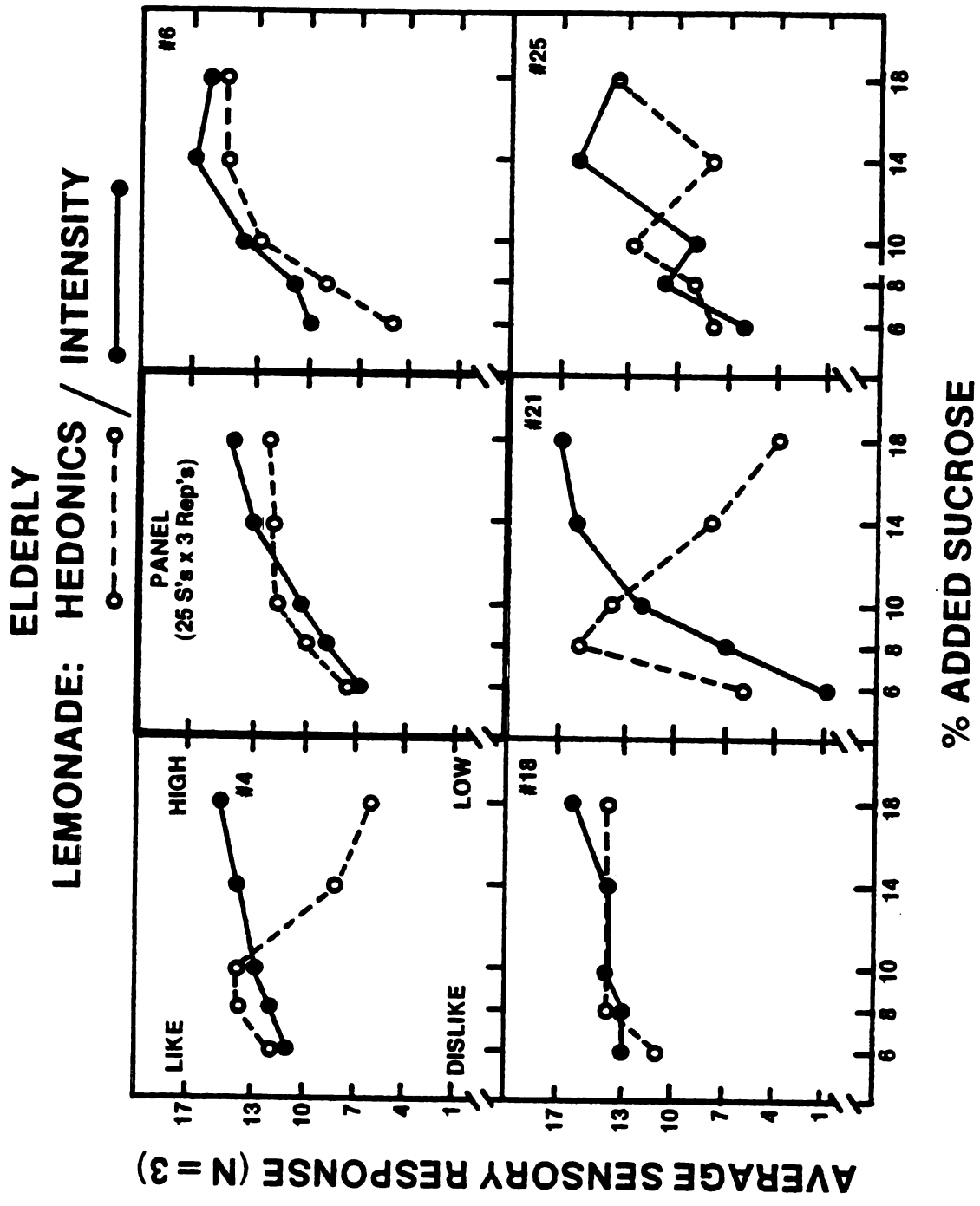

ELDERLY
LEMONADE: HEDONICS / INTENSITY
PANEL
(25 S's x 3 Rep's)
#4
#6
#18
#21
#25
HIGH
LOW
LIKE
DISLIKE
AVERAGE SENSORY RESPONSE (N = 3)
% ADDED SUCROSE

Figure 6. Individual sensory graphs of five response types to lemonade intensity and hedonics by the college subjects in Experiment I.2.

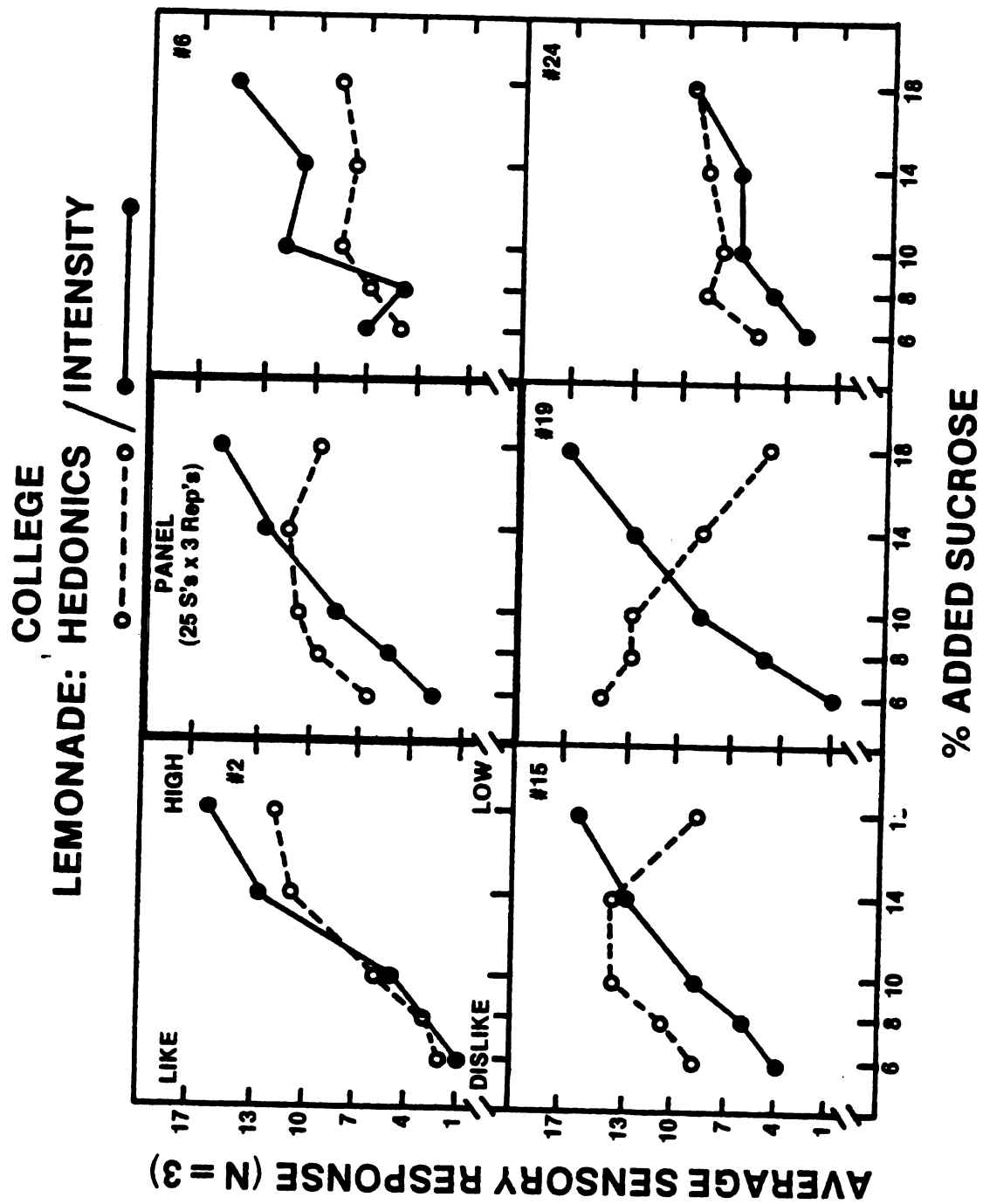

COLLEGE
LEMONADE: HEDONICS / INTENSITY
PANEL
(25 S's x 3 Rep's)
HIGH
LOW
LIKE
DISLIKE
#2
#6
#15
#19
#24
AVERAGE SENSORY RESPONSE (N = 3)
% ADDED SUCROSE

Table (24). Elderly subjects (identified by #'s) who either displayed flat or confused sensory functions in Experiment I.1. and 2.

INTENSITY SCALING (elderly only)							
Flat Function[1]				Confused Function[2]			
Tom. Juice/Lemonade				Tom. Juice/Lemonade			
# 8*	#1	#13		# 1*	#13*	#1	#13
#11	#2	#14		# 2*	#15	#2	#14
#13*	#3	#17		# 8*	#20*	#6	#20
#18*	#4	#18		#10	#24	#7	#22
#20	#8	#22		#11	#25*	#8	#25

FLAT FUNCTION[3] FOR BOTH HEDONIC AND INTENSITY	
Tomato Juice	Lemonade
#13	#17
#18	#18

[1] Three data points within 2 intensity points of each other (1-17 scale).
[2] Incorrect scaling of the order of intensity.
[3] Horizontal scaling of intensity and hedonics within the individual.

* same individual for both tomato juice and lemonade.

The next question one might pose is if any subjects had flat sensory slopes for both hedonics and intensity within the same sensory system. Two horizontal slopes would be highly suggestive of a decreased taste acuity in the elderly.

Inspection of the individual sensory responses found only two individuals who truly possess flat functions for tomato juice (#13, #18), and two for lemonade (#17, #18), although #18 is the same subject in both food systems (Table 24).

Table (25) compares the number of elderly and college students with flat or confused sensory functions in Experiment I. As previously noted, few college students demonstrated any difficulty in the sensory separation of tastant concentrations. Three college subjects, however, possessed confused sensory functions for lemonade intensity. In each case, only one concentration of sucrose was incorrectly scaled in proper sequence.

Table (25). Comparison of the number of subjects with flat or confused sensory functions in Experiment I.1. and 2.

TOMATO JUICE (INTENSITY)			
Flat Function[1]		Confused Function[2]	
Elderly	College	Elderly	College
5	0	10	0

LEMONADE (INTENSITY)			
Flat Function		Confused Function	
Elderly	College	Elderly	College
10	0	10	3

[1]A function where three or more concentrations are rated within 2-points of each other--horizontal taste intensity function.

[2]A function where the subjects scale one or more concentrations incorrectly, i.e., rate 10% sucrose as being sweeter than 14%.

A.2. Experiment I, Part 3. Odor Identification of Ten Food Odorants.

Figure 7 represents the results of the odor identification study in which the elderly and college students attempted to identify ten common food odorants by olfaction alone. The obvious disparity in the percent correct response for each of the ten food odorants was quite unexpected. For the entire set of foods, the elderly averaged a percent correct response of 33.5 vs. 86.4% for the college students.

Table (26) contains the percent correct response in the odor identification experiment. The actual analysis of the significance of these odor recognition studies varies according to which author is consulted. Thus, in fairness to the data, the results of Experiment I, Part 3 were subjected to three separate statistical procedures. Fortunately, in each case, the evaluation is the same--that the elderly and college students are definitely different in their ability to recognize food odors.

Figure 7. Percent correct response to the identification of the ten food odorants by the college and elderly subjects, Experiment I.3.

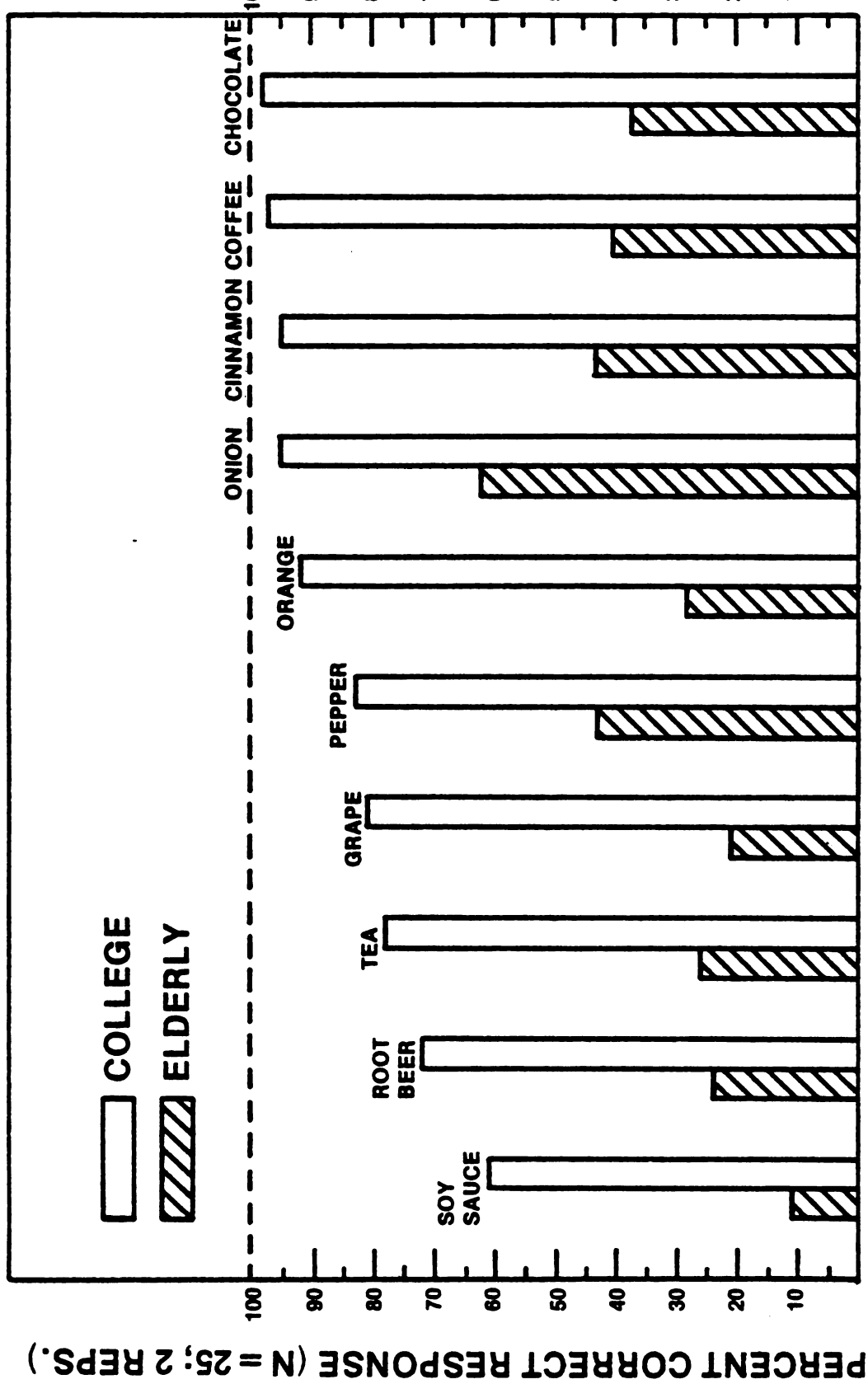

ODOR IDENTIFICATION: COLLEGE vs. ELDERLY
COLLEGE
ELDERLY
SOY SAUCE
ROOT BEER
TEA
GRAPE
PEPPER
ORANGE
ONION
CINNAMON
COFFEE
CHOCOLATE
100
90
80
70
60
50
40
30
20
10
PERCENT CORRECT RESPONSE (N = 25; 2 REPS.)
AVERAGE SCORE OF TEN FOOD ODORANTS

Table (26). Results of the odor identification study (percent correct response) between the elderly and college students.[1]

ODOR RECOGNITION--EXPERIMENT I, PART 3

	College				Elderly			
% correct response								
Foods	Reps:			$\bar{X}$	Reps:		$\bar{X}$	
	1	2	3	(mean)	1	2	(mean)	3
Soy sauce	54	68	68	63	13	9	11	27
Pepper	82	83	85	83	47	38	43	50
Grape	81	81	86	83	24	17	21	58
Cocoa	96	100	100	99	23	50	37	69
Tea	74	81	88	81	24	28	26	31
Root Beer	68	75	79	74	24	23	24	58
Coffee	97	97	97	97	35	44	40	46
Cinnamon	92	97	100	96	36	50	43	45
Orange	90	94	94	93	27	28	28	42
Onion	90	100	96	95	55	69	62	77
$\bar{X}$ =	82	88	89	86	31	36	34	52
S.D. =	14	11	10	12	13	18	14	16
χ^2 =	20.5	13.4	10.6		46.2	82.4		56.3

[1]The third trial of the elderly odor identification was completed with a list of foods in the taste booth.

χ^2=Chi-square values all significant at the $p<0.001$ level. (Foods were judged sign. different from each other).

Part 2. Odor Identification with a List, in the Elderly. Experiment I.3.

In the third replication for the elderly, a list of the actual food items was placed inside the taste booth for their inspection. The intention was that if memory was the major factor in the previously low recognition scores, the list would increase the olfactory recognition and the scores might show improvement.

Figure 8 displays the percent correct response to the ten food odorants, before and after the list. The scores without the list are the average of the first two trials, and the scores with a list are the scores of one trial only (the third). The actual food list contained the foods used with the addition of six more. The six additional food items lowered the odds of a blind guess to 1/16 or six percent.

The addition of the list did not increase the recognition scores as much as anticipated--from a mean score of 34 percent to 52 percent. If one subtracts the 6% chance to select the odor by blind luck, then the score narrows to 34 vs. 46 percent. Table (26) contains the list given scores as well as the "without" list scores by the elderly.

The odor identification graph, Figure 8, is arranged so that, as one goes from left to right, the improvement for each food odor increases. The ability to recognize chocolate, root beer and grape increased significantly with the list, while the common items such as pepper and onion increased only slightly. Chi-square analysis (χ^2=189.19) found the new frequencies significantly different than

that of the score without a list. Use of the paired t-test established the increased percent response to be highly significant ($t=4.97$, $p<0.001$), although not high enough to approach the recognition scores of the college students. As noted earlier, the students, by the third trial, had increased their scores to 89 percent from the first trial of 82 percent.

Figure 8. The percent correct response to the ten food odorants, with and without a list, by the elderly of Experiment I.3.

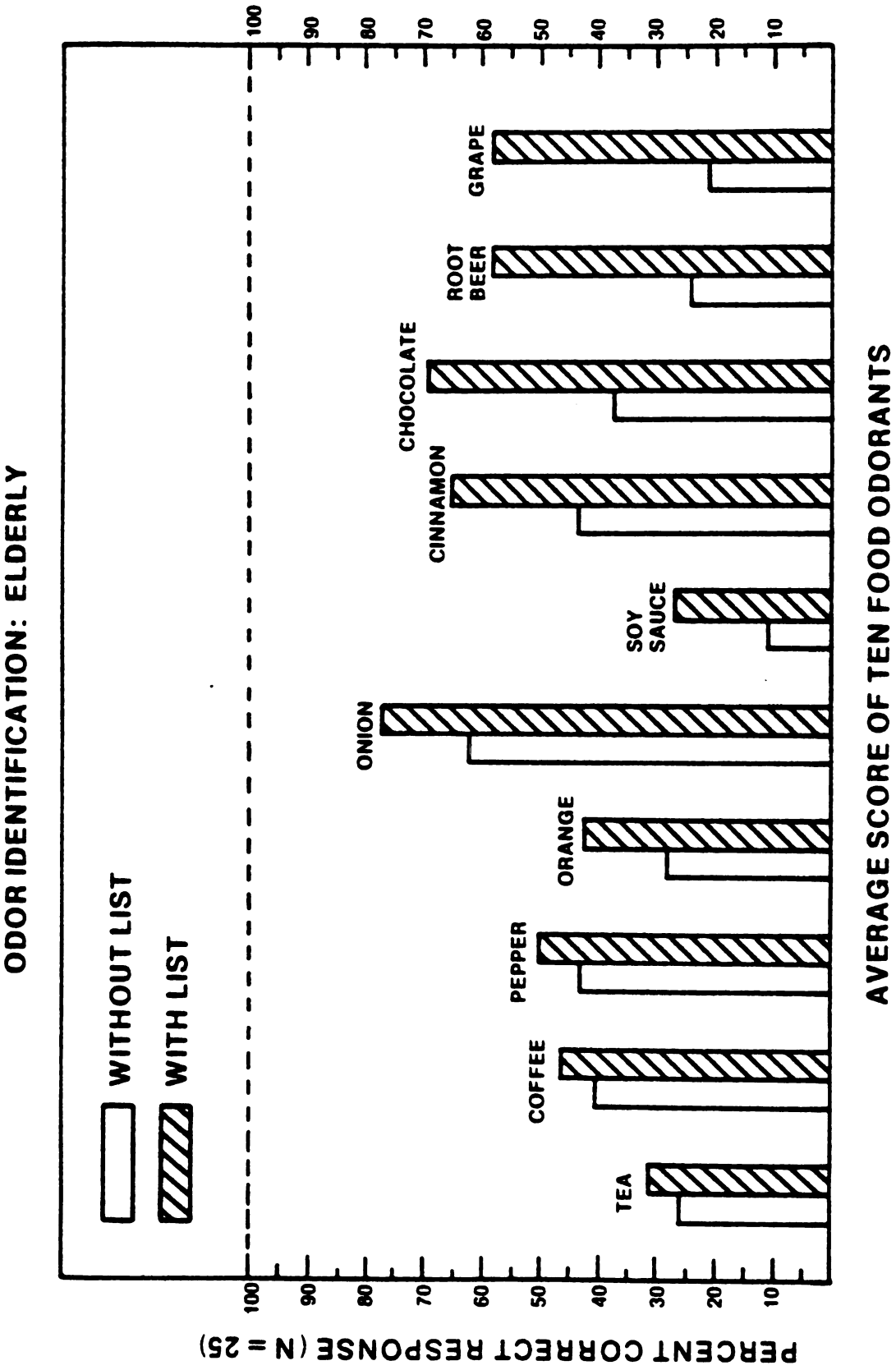

ODOR IDENTIFICATION: ELDERLY
WITHOUT LIST
WITH LIST
PERCENT CORRECT RESPONSE (N = 25)
100
90
80
70
60
50
40
30
20
10
TEA
COFFEE
PEPPER
ORANGE
ONION
SOY SAUCE
CINNAMON
CHOCOLATE
ROOT BEER
GRAPE
AVERAGE SCORE OF TEN FOOD ODORANTS

Table (27). Statistical treatment of the odor recognition of Experiment I.3.

A. Chi-Square:

$\chi^2 = 33.3^{***}$

Reference: Hewlett-Packard (1974)

B. Paired t-statistic:

T = 16.55,*** df=9

Reference: Roessler (1975)

C. Analysis of Variance:

	DF	SS	MS	F
Total	39	34310.0		
Odors	9	5160.5	573.39	6.81***
Groups	1	26832.4	26832.4	318.49***
Reps	9	632.2	70.24	0.83
Error		1684.90	84.25	

Reference: Roessler (1975)

***, Significant at P<0.001.

A.3. Experiment I, Part 3. Intensity Ratings to Seven Food Odorants by the Elderly and College Students.

Figure 9 depicts the average intensity response to seven food odorants by the 25 elderly and 25 college students. Odorants are arranged such that the intensity differences between the two groups increase as one visually travels to the right.

The food odorants--cocoa, tea and grape--received similar odor intensities by both groups. However, two very common foodstuffs, pepper and coffee, were rated quite dissimilar. Surprisingly, pepper, known to have a strong trigeminal or pain stimulus component, was rated at almost half the strength of the college students' level by the elderly participants. This difference agrees with the previous observations by the experimenters which found that at least 20% of the elderly failed to detect the food reference for high odor intensity vinegar.

In any event, the intensity rankings of the odorants by the two groups were not related, as the Spearman rank correlation coefficient is only r_s=.643. The nested analysis of variance (Table 28) finds significant variation in the group means (41.8 vs. 61.4), the odorants, and judges. Replications, however, were not significantly different, indicating good agreement by all judges through the three repeat trials. The group by odorant interaction was highly significant, indicating the lack of agreement for intensity ratings of the seven odorants by the elderly and college students. Inspection of Table (29) reemphasizes the difficulty of the elderly to differentiate the seven food odorants. Using the Scheffé mean comparison technique, a very

conservative separation technique, the elderly failed to separate many of the odorants by intensity alone. One reason for this failure to differentiate is the greater inherent error variance in their intensity judgments. The separate AOV of Table (30) reveals a significant replication factor and a significant odorant by replication factor. In addition, note the large error variance for the elderly as compared to the college students (M.S.: 242.06 vs 157.79).

Figure 9. Average odor intensity of seven food stimuli, perceived by the college vs. the elderly of Experiment I.4.

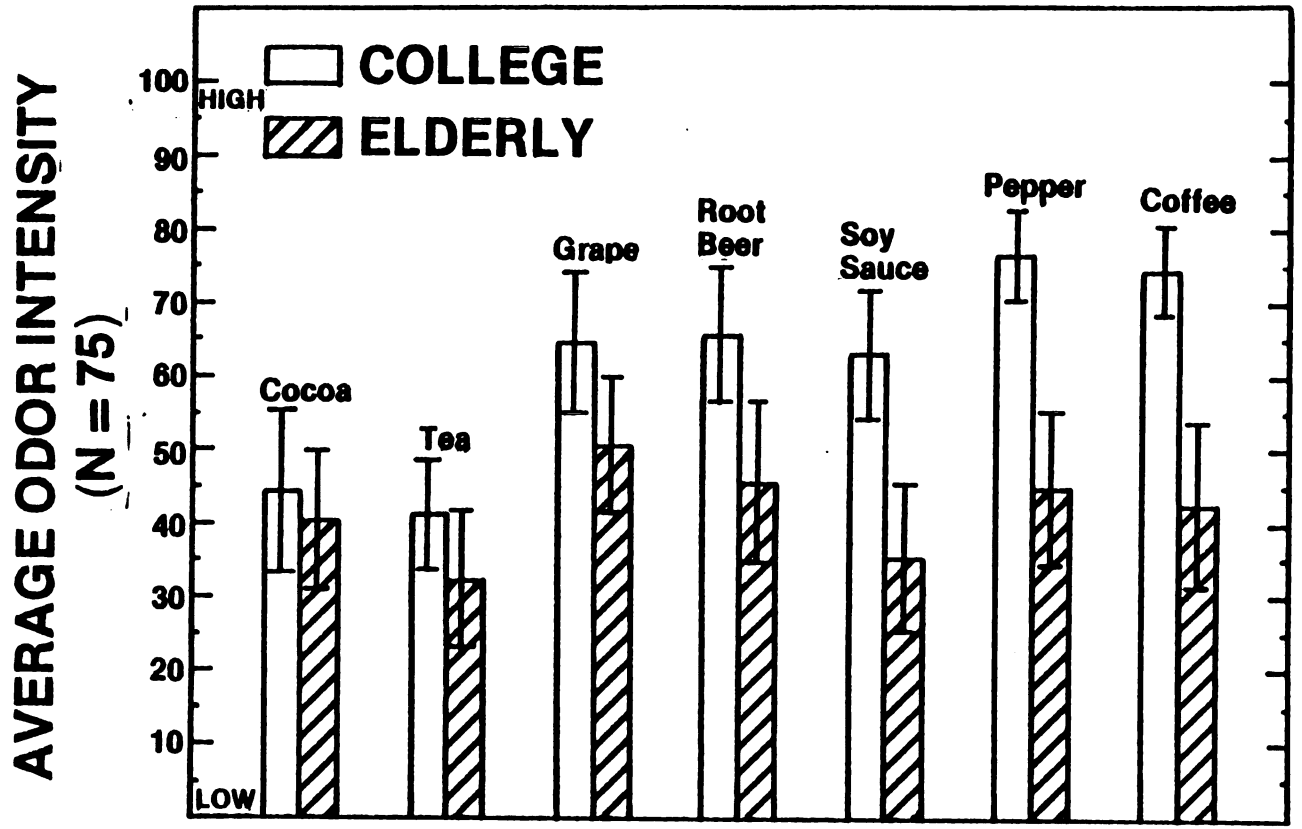

COLLEGE
ELDERLY
AVERAGE ODOR INTENSITY
(N = 75)
HIGH
LOW
100
90
80
70
60
50
40
30
20
10
Cocoa
Tea
Grape
Root Beer
Soy Sauce
Pepper
Coffee
SEVEN OLFACTORY STIMULI

Table (28). Nested AOV of the intensity responses to the seven food odorants by the elderly and college students of Experiment I.4.

Source of Variation	D.F.	S.S.	M.S.	F-ratio	Test Source[1]
A-Groups	1	100040.0	100040.0	26.23***	C
B-odorants	6	73721.5	12286.9	25.26***	BC
C-judges	24	91546.	3814.2	9.58***	Error
D-replications	2	1697	848.5	1.62	CD
A X B	24	130615	4749.25	9.76***	BC
B X C	144	70044.5	486.42	1.22	Error
A X D	2	6980.5	3490.25	7.18**	BC
C X D	12	7272.5	522.97	1.31	Error
B X D	48	25102.5	606.04	1.52	Error
A X C X D	48	17347.0	361.4	0.91	Error
ERROR	792	315193.5	397.97		
TOTAL	1049				

[1]The Source of the denominator used to calculate the tabulated F-ratio.

, *, Significant at P<0.01 and 0.001, respectively.

Table (29). Intensity means for groups and odorants of the seven food odorants, Experiment I.4. [1]

FOOD ODORANT INTENSITY

Nested AOV:

A-Groups:***

Elderly	College
41.81	61.44

B-Odorants: ***

Elderly

Tea	Cocoa	Soy S.	Grape	Root B.	Coffee	Pepper
41.20	44.52	63.6	64.72	65.72	74.36	76.40

Separate AOV:

Elderly

Tea	Soy S.	Cocoa	Coffee	Pepper	Root B.	Grape
32.56	35.44	40.52	42.60	44.92	45.84	50.80

College

Tea	Cocoa	Soy S.	Grape	Root B.	Coffee	Pepper
41.20	44.52	63.16	64.72	65.72	74.36	76.40

[1]Within the same row, means underscored by the same line are not significantly different.

***, Significantly different at P<0.001.

Table (30). Separate AOV for ratings of aroma intensity, and to the amount of salivation, in the elderly and college students of Experiment I.4. and 5.

Source of Variation	D.F.	Study I, Part 4. Odor Intensity Elderly F	Odor Intensity College F	D.F.	Study I, Part 5. Salivation Elderly F	Salivation College F
A-Levels	6	12.198***	89.632***	3	152.321***	263.075***
B-Subjects	24	26.039***	18.967	24	39.459***	37.937***
C-Replications	2	15.615***	2.930	2	2.428	4.276*
A X B	144	1.507*	3.075***	72	2.772***	3.581***
A X C	12	2.190*	2.393**	6	0.652	1.724
B X C	48	2.333***	2.017***	48	4.494***	5.224***
A X B X C	288	242.06[1]	157.799	144	0.1521	0.1514
TOTAL	524			299		

*, **, ***, Significant at $P<0.05$, 0.01, and 0.001, respectively.

[1]Mean sum of squares.

A.4. Experiment I, Part 4. Salivary Flow Rates to Food Stimuli in the Elderly and College Students.

Figure 10 illustrates a comparison of the whole mouth salivary flow rates to sniffing lemons and tasting lemonade between the elderly and college students. Salivary resting levels were lower in the elderly than the college students, but not significantly so. Of greater interest was the increased salivary flow rates to the lemons by the college students, whereas the elderly demonstrated only a slight increase. Likewise, the elderly averaged only 80% of the flow rate to lemonade as compared to the college students.

The nested AOV (Table 31) reveals that, while the judges and treatments differed significantly, the overall group mean and the replication did not. The interaction of greatest importance, the A X C or group by treatment interaction, was significantly different, indicating that the elderly did not follow the same pattern of increased salivation to the food stimuli as the college students.

The separate AOV (Table 30) compares the variances of each group. Both groups demonstrated significant levels of variation in the concentration and subject factors. The elderly, however, were a bit more uniform across replications than the college students, as evidenced by a non-significant F-ratio.

By use of the mean separation techniques of Scheffe' and Least Significant Difference (LSD), one may note from Table (32) that the elderly did not significantly increase their salivation levels when sniffing the lemons, while the college students did so (using the

LSD method). The Scheffé test is considered the most stringent mean comparison technique, while the LSD is the least sensitive. A comparison and analysis of the two methods is presented with great clarity by Keppel (1973). Both methods were illustrated in the interest of accurately assessing and evaluating the salivation data, although, in this case, the two techniques came to the identical conclusion.

Figure 10. Average whole-mouth salivary secretion to sniffing and tasting lemons in the elderly and college students in Experiment I.5.

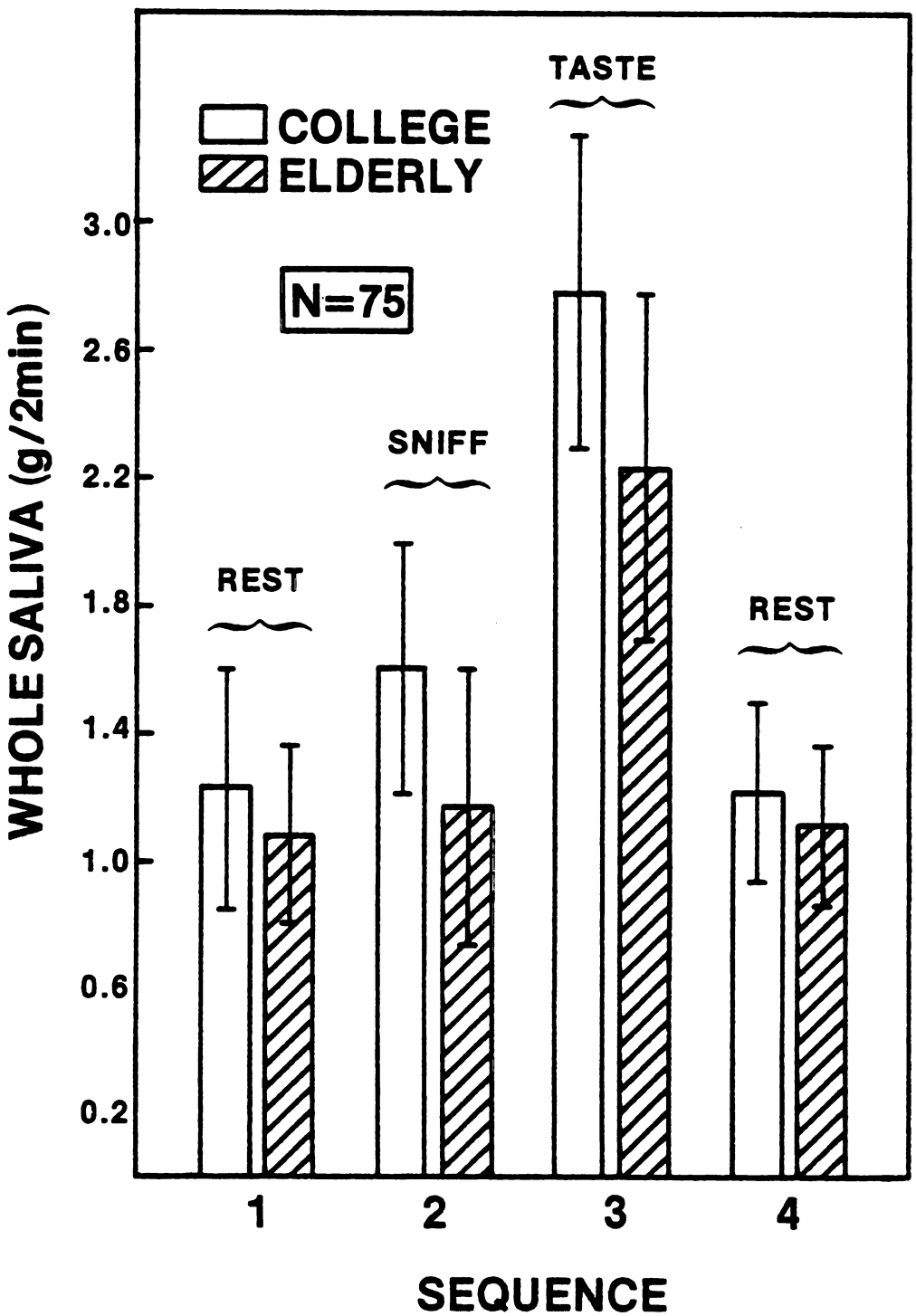

TASTE
COLLEGE
ELDERLY
N=75
SNIFF
REST
REST
WHOLE SALIVA (g/2min)
3.0
2.6
2.2
1.8
1.4
1.0
0.6
0.2
1
2
3
4
SEQUENCE

Table (31). Nested AOV of the salivation rates to food stimuli between the elderly and college students in Experiment I.5.

Source of Variation	D.F.	S.S.	M.S.	F-ratio	Test Source[1]
A-Groups	1	14.07	14.07	3.30	B
B-Judges	48	204.84	4.27	6.78***	Error
C-Concentrations	3	184.18	61.39	219.25***	BC
D-Replications	2	1.88	0.94	2.29	BD
A X C	3	4.81	1.60	5.71**	BC
B X C	144	39.85	0.28	0.44	Error
A X D	2	0.16	0.08	0.29	BC
B X D	96	38.95	0.41	0.65	Error
C X D	6	1.81	0.30	0.48	Error
A X C X D	6	0.35	0.06	0.10	Error
ERROR	288	182.13	0.63		
TOTAL	599				

[1]The Source of the denominator used to calculate the tabulated F-ratio.

,*, Significant at P<0.01, and 0.001, etc.

Table (32). Mean separation methods of Scheffé and LSD on the salivation data of the elderly and college students, Experiment I.5. [1,2]

Whole Saliva (g/2 min)							
Elderly (using Scheffé)				College (using Scheffé)			
Sequence:				Sequence:			
-1-	-4-	-2-	-3-	-4-	-1-	-2-	-3-
1.08	1.11	1.16	2.22	1.21	1.23	1.59	2.77
Using LSD:				Using LSD:			
-1-	-4-	-2-	-3-	-4-	-1-	-2-	-3-
1.08	1.11	1.16	2.22	1.21	1.23	1.59	2.77

[1] 1=First resting, 2=Sniffing, 3=Taste, 4=Final resting.

[2] Underlined values within a row do not differ significantly.

A.5. Experiment I, Part 5. Nutrient Analysis of the 3-day dietary recalls.

College Students

Table (33) lists the means, standard deviations, minimum and maximum values, and percent 1974 RDA for the 25 students. Generally, the college subjects met or exceeded the RDA for eleven nutrients: total protein, vitamins A, C, B_1, B_2 , niacin, iron, calcium, phosphorus and magnesium. Nutrients falling short of the RDA were total calories, pyridoxine, folacin, vitamin D, and zinc. Copper levels were 1.39 mg/day, somewhat below the suggested level of 2 mg/day.

Elderly Subjects

Table (34) lists the means, standard deviation, minimum and maximum values, and percent 1974 RDA for the 25 elderly subjects. Eleven nutrients met or exceeded their 1974 RDA's: total kilocalories total protein, vitamins C, B_1, B_2, niacin, B_{12}, and A, iron, calcium and phosphorus. Five nutrients which failed to reach the 100 percentile of the RDA were B_6, folacin, vitamin D, magnesium and zinc. Total zinc, (10.35 mg), although substantially less than the recommended allowance of 15 mg, was marginally higher than the students' diets.

Only two nutrients differed between the two groups: total calories was a bit lower in the college students (86.99 vs. 102.3% RDA) and magnesium levels were a bit lower for the elderly (87.23 vs. 100.34% RDA).

Table (33). Nutrient analysis of the 3-day dietary recall of the college students, Experiment I. [1,2]

	COLLEGE STUDENTS					
Nutrient	$\bar{x}$	S.D.	Min.	Max.	1974 % RDA	Units
Energy	2142.04	885.24	765.26	4255.65	86.99	kcal
Protein	83.66	39.62	27.54	193.92	159.65	g
Fat	82.12	39.82	21.11	183.99	---	g
Carb.	268.69	109.11	97.25	526.75	---	g
Cholesterol	311.15	180.87	59.02	822.48	---	mg
Crude fiber	4431.62	2325.12	918.44	10441.84	---	mg
Vitamin C	172.87	103.49	10.84	436.35	384.14	mg
Thiamin	1.43	0.83	0.33	3.97	110.97	mg
Niacin	21.55	11.56	5.31	58.84	134.32	mg
Riboflavin	2.07	1.32	0.57	5.79	135.69	mg
Pyridoxine	1.43	0.89	0.47	4.55	71.47	mg
Vitamin B_{12}	3.39	2.55	0.42	12.59	112.98	µg
Folacin	300.35	153.99	23.21	740.39	75.08	µg
Vitamin A	9439.03	8784.33	2257.49	33795.96	213.27	IU
Vitamin D	121.10	142.99	0.21	628.94	30.27	IU
Iron	14.02	7.21	5.40	40.16	114.43	mg
Calcium	1037.48	670.78	237.08	2919.94	129.68	mg
Phosphorus	1395.73	688.16	397.16	3167.77	174.46	mg
Sodium	3084.10	1349.38	631.85	5912.21	---	mg
Potassium	3344.07	1456.91	1399.71	7609.21	---	mg
Magnesium	327.49	218.51	86.05	1050.72	100.34	mg
Copper	1.387	0.711	0.453	2.729	69.35	mg
Zinc	9.676	6.29	3.21	32.98	64.50	mg
Sugars	132.44	62.31	47.49	292.14	---	g

[1] $\bar{x}$: mean, S.D.: standard deviation; Min.: minimum value, Max: maximum value.

[2] Value for copper (2 mg) is considered reasonable.

Table (34). Nutrient analysis of the 3-day dietary record of the elderly, Experiment I. [1,2]

	ELDERLY					
Nutrient	$\bar{x}$	S.D.	Min.	Max	1974 %RDA	Units
Energy	1923.55	272.22	1489.51	2406.82	102.29	kcal
Protein	80.42	16.23	54.39	113.96	167.01	g
Fat	73.31	15.15	51.45	111.04	----	g
Carb.	247.06	41.02	185.72	320.37	----	g
Cholesterol	367.01	150.47	135.85	687.48	----	mg
Crude fiber	4124.23	816.6	2745.7	6003.2	----	mg
Vitamin C	115.97	31.33	60.0	183.53	257.7	mg
Thiamin	1.27	0.33	0.830	2.200	118.4	mg
Niacin	20.84	5.32	11.99	32.12	154.80	mg
Riboflavin	2.65	0.976	1.01	4.42	215.66	mg
Pyridoxine	1.67	0.52	0.87	3.12	83.63	mg
Vitamin B_{12}	15.54	12.57	1.76	31.33	517.94	µg
Folacin	277.65	91.98	185.35	602.84	69.41	µg
Vitamin A	16648.77	9895.38	4136.15	31474.16	409.44	IU
Vitamin D	217.27	134.47	18.72	506.92	54.31	IU
Iron	16.63	4.17	9.51	27.01	166.30	MG
Calcium	855.75	314.2	315.73	1474.52	106.96	MG
Phosphorus	1341.91	300.18	808.92	1834.27	167.74	mg
Sodium	2407.1	487.39	1488.15	3466.55	----	mg
Potassium	3266.67	550.65	2143.99	4081.78	----	mg
Magnesium	264.94	47.55	2261.26	355.27	87.23	mg
Copper	1.884	0.698	0.630	3.19	94.00	mg
Zinc	10.35	2.36	6.65	14.81	68.99	mg
Sugars	141.07	33.60	85.32	196.42	----	g

[1] $\bar{x}$: mean, S.D.: standard deviation; Min.: minimum value, Max: maximum value.

[2] Value for copper (2 mg) is considered reasonable.

A.6. Experiment I, Part 6. Hair Analyses for Zinc/Copper in the Elderly and College Students.

Table (34B) contains the results of the hair analyses for zinc/copper in all subjects participating in Experiment I, II.

The college students averaged 214.5±61 ug/g of zinc and 39.2±19 ug copper per gram of hair; while the elderly's values were somewhat lower with 188.3±47 ug/g of hair for zinc and 27.5±12 ug/g copper. The zinc/copper values for one elderly subject were lost due to a hand-eye coordination error by this author, allowing the sample to empty onto the floor.

Statistical correlations were attempted between the hair concentrations of zinc and copper for both the university students and elderly classifications. The correlation between copper (x) and zinc (y) levels in the hair of university students was: r=.02, while the similar correlation for the elderly group was: r=.58.* The significant positive correlation of the concentrations of zinc and copper in hair for the elderly signifies that higher levels of zinc are usually associated with higher levels of copper. It is interesting to note that this relationship did not exist in the younger university age population. Analysis of variance revealed NSD between the elderly and college in hair zinc (F=2.79), but significant in the copper levels at p.05 (F=6.92).

Table (34B). Zinc/copper analyses of hair samples from the college-age and elderly subjects, Experiment I and II.

	College		Elderly	
Subject	Zn	Cu	Zn	Cu
1	212	22 ug/g [1]	187	13
2	241	27	175	37
3	310	64	201	41
4	170	57	209	19
5	202	40	117	23
6	187	45	187	29
7	117	97	219	37
8	199	46	330	61
9	232	29	163	19
10	404	31	147	13
11	312	71	113	19
12	208	41	193	27
13	117	52	214	33
14	151	17	253	29
15	198	33	188	47
16	183	18	137	23
17	179	37	241	19
18	246	41	214	30
19	196	27	*	*
20	237	40	163	22
21	246	46	159	27
22	163	22	140	19
23	197	19	214	17
24	213	23	193	41
25	242	36	161	14
	214.5±61	39.2±19	188.3±47	27.5±12

[1]All values in micrograms/gram hair sample.

*Data lost in analyses.

B. Experiment II, Parts A and B. Odor identification in the elderly, with (Part A) and without (Part B) a list.

Figure 11 compares the percent correct response to odor identification of eight food odorants and two blanks by 25 elderly subjects. In Experiment II, two of the odorants were the same (grape and pepper) while the other six odorants were common household foodstuffs.

The results of Experiment II were similar to the odor recognition studies of Experiment I. Elderly subjects averaged 22% and 30%, without and with the list, in contrast to the 75% and 90% recognition performance of the college students.

Surprisingly, the elderly increased their recognition scores only 8% above the without list trial. And, if one discounts 6% due to chance guessing alone, the improvement of 2% seems incredibly small. Garlic powder recognition scores actually decreased when subjects used the list (20% to 27%). In contrast, with the aid of the list of food odorants, the college students were able to increase their overall scores by 15% to a 90% level (Figure 12). Lemon and pepper received 100% recognition in the college students, while the best recognized food odors for the elderly were the same, but with 42% recognition values. The least volatile food odor, maple syrup, was the most difficult to recognize for both groups.

Figure 11. Percent correct response to the odor identification of 8 food odorants and 2 blanks, by the elderly of Experiment II.A, B.

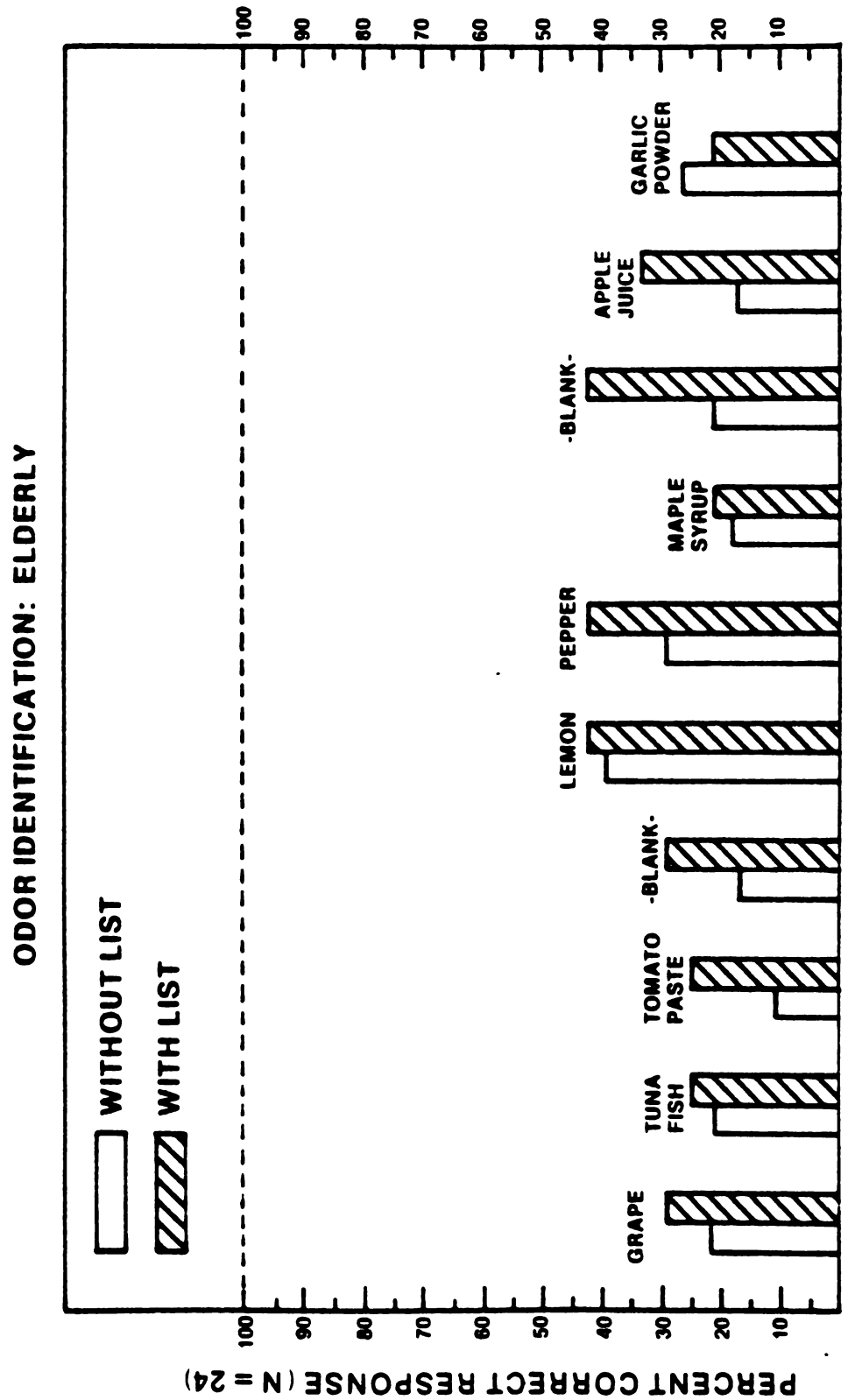
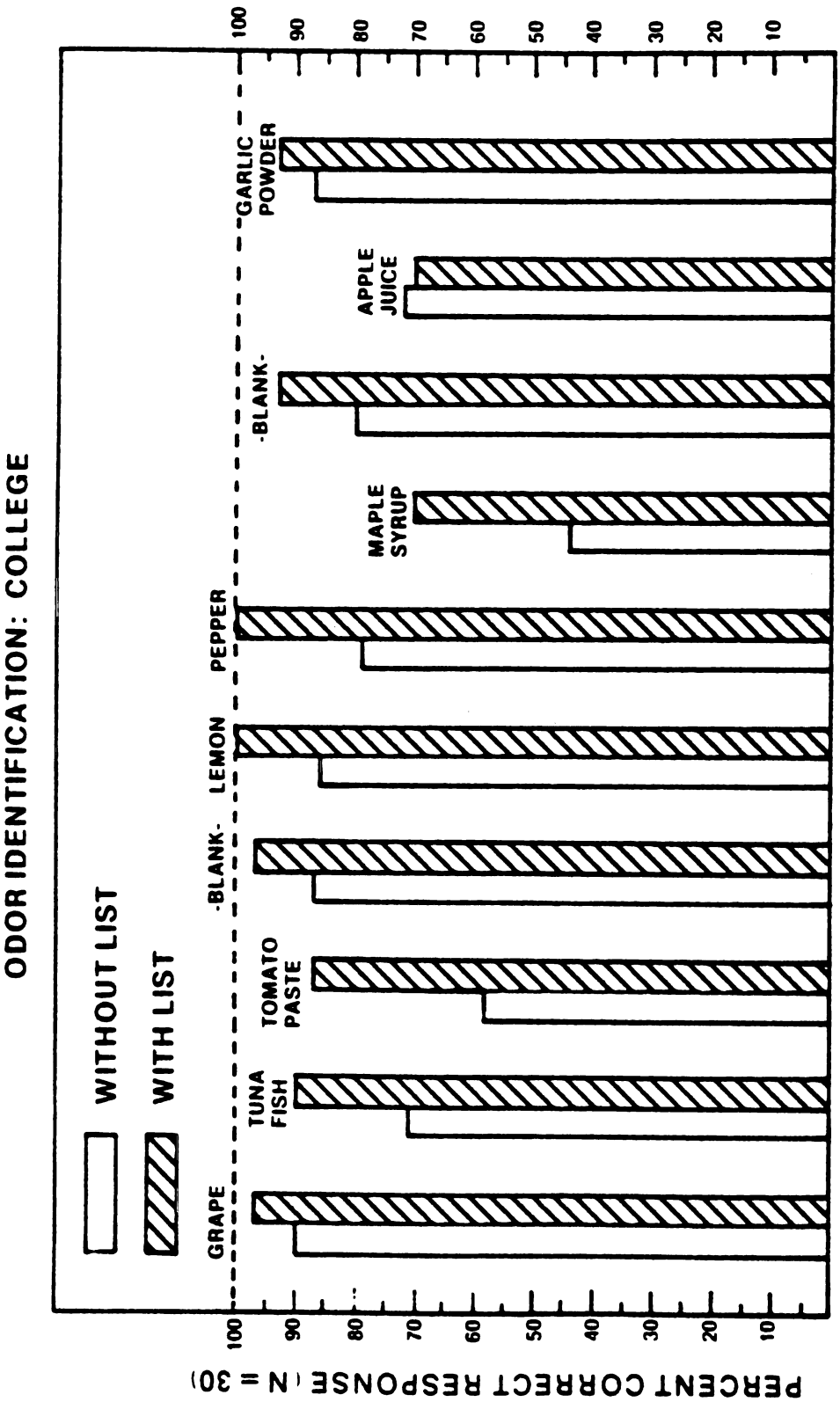

ODOR IDENTIFICATION: ELDERLY
WITHOUT LIST
WITH LIST
PERCENT CORRECT RESPONSE (N = 24)
100
90
80
70
60
50
40
30
20
10
GRAPE
TUNA FISH
TOMATO PASTE
-BLANK-
LEMON
PEPPER
MAPLE SYRUP
-BLANK-
APPLE JUICE
GARLIC POWDER
AVERAGE SCORE OF 8 FOOD ODORANTS AND 2 BLANKS

Figure 12. Percent correct response to the odor identification of 8 food odorants and 2 blanks by the college students of Experiment II.A, B.


ODOR IDENTIFICATION: COLLEGE
WITHOUT LIST
WITH LIST
PERCENT CORRECT RESPONSE (N = 30)
100
90
80
70
60
50
40
30
20
10
GRAPE
TUNA FISH
TOMATO PASTE
-BLANK-
LEMON
PEPPER
MAPLE SYRUP
-BLANK-
APPLE JUICE
GARLIC POWDER
AVERAGE SCORE OF 8 FOOD ODORANTS AND 2 BLANKS

As Table (35) illustrates, the improvement in odor recognition by the use of a list of foods was significant for both groups, although one must interpret the elderly data very cautiously. The Chi-square (χ^2) analysis of each column was highly significant. This demonstrates that the food odorants were not equally recognizable within the group.

Figures 13 and 14 directly compare the odor identification for the eight food odorants and two blanks, without the list and with the list. The disparity between the two groups is especially great for grape and garlic powder. In addition, the college students were adept at identifying the blank samples in the first trial--especially since the subjects were not told there were two blank samples.

A further analysis of the food odors given to the blank samples may be of interest. In Experiment II.A, (without a list of food odorants), 84% of the college subjects recognized that the two samples were blank. Table (36) contains the variety of responses written into the blank food spots. The foods apparently do not classify themselves into easily recognizable categories. It is interesting to note, however, that the blank preceded by tomato paste elicited guesses with decidedly meat-like properties, while the blank preceded by maple syrup elicited more fruit-like responses. As for the elderly, they were more conservative in their guesses, with fruits being the predominant choice (see Table 37).

Another important question yet unanswered is the problem of the effect of age on odor recognition scores. As previously described in the methods, the total possible score for seven odorants at three points each is 21.

Figure 13. Comparison of the odor identification scores without the list, by the elderly and college subjects, Experiment II.A.

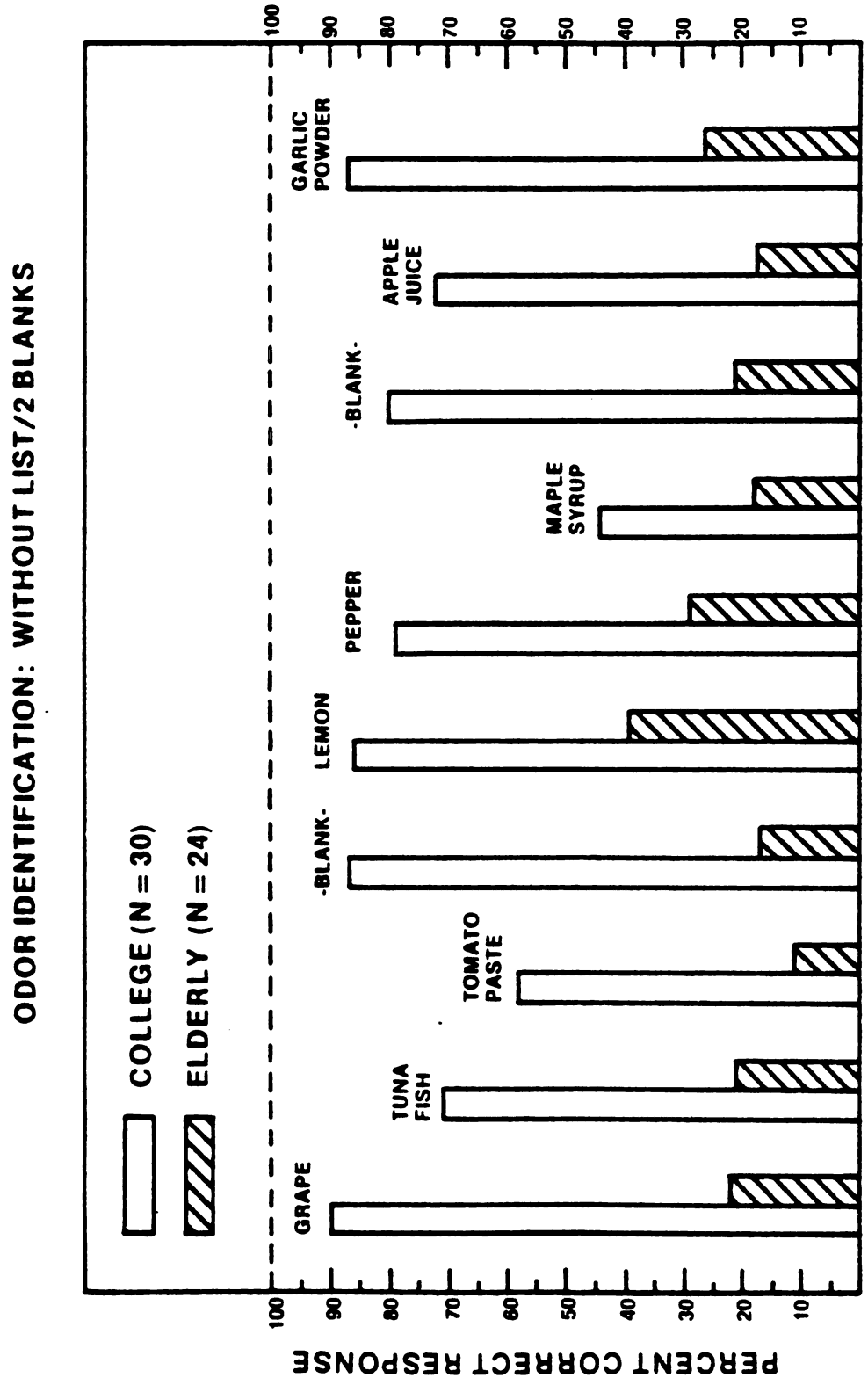

ODOR IDENTIFICATION: WITHOUT LIST/2 BLANKS
COLLEGE (N = 30)
ELDERLY (N = 24)
PERCENT CORRECT RESPONSE
100
90
80
70
60
50
40
30
20
10
GRAPE
TUNA FISH
TOMATO PASTE
-BLANK-
LEMON
PEPPER
MAPLE SYRUP
-BLANK-
APPLE JUICE
GARLIC POWDER
AVERAGE SCORE OF 8 FOOD ODORANTS AND 2 BLANKS

Figure 14. Comparison of the odor identification scores, with the list, by the elderly and college subjects, Experiment II.B.

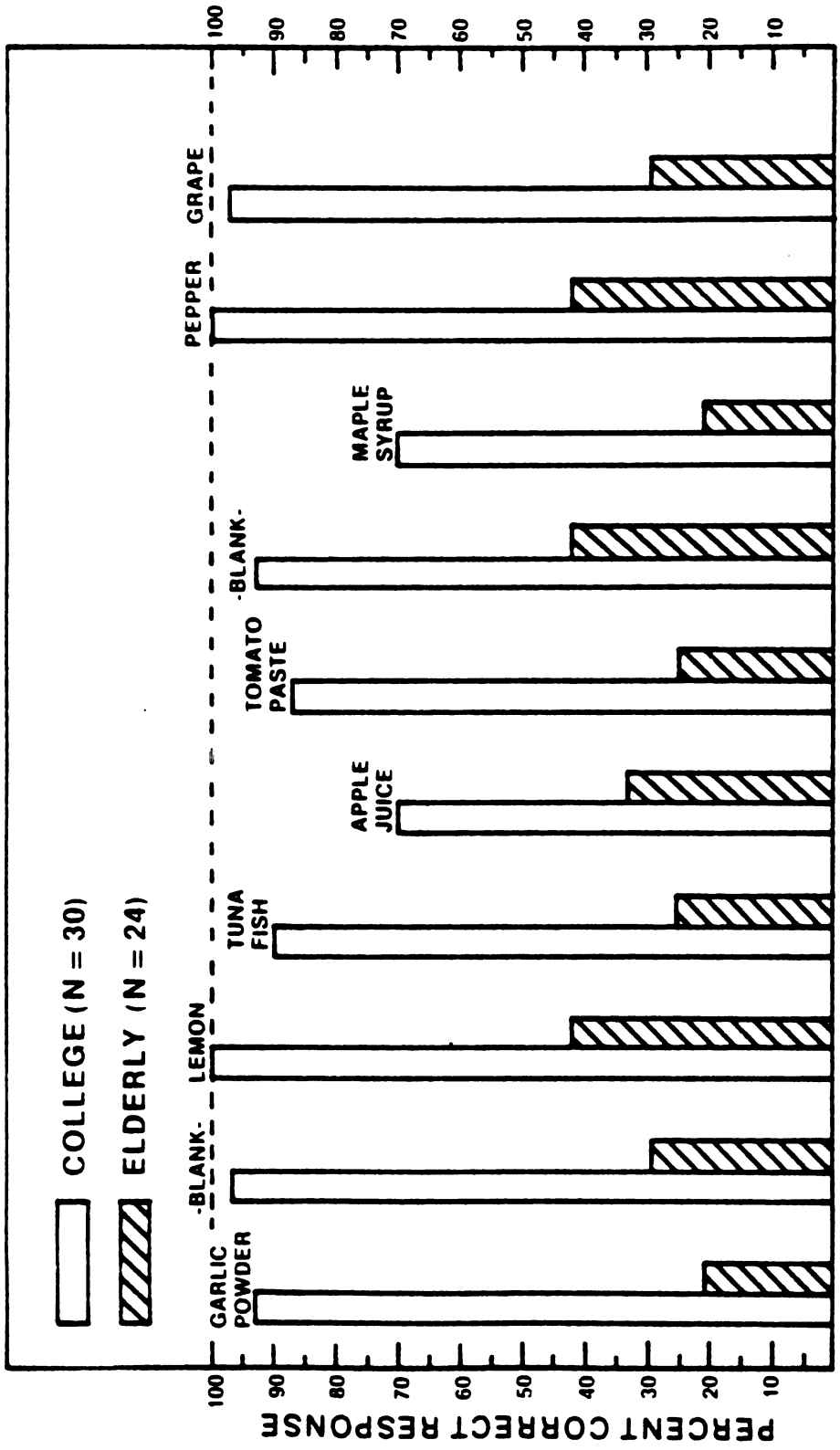

ODOR IDENTIFICATION: WITH LIST/2 BLANKS
COLLEGE (N = 30)
ELDERLY (N = 24)
PERCENT CORRECT RESPONSE
100
90
80
70
60
50
40
30
20
10
GARLIC POWDER
-BLANK-
LEMON
TUNA FISH
APPLE JUICE
TOMATO PASTE
-BLANK-
MAPLE SYRUP
PEPPER
GRAPE
AVERAGE SCORE OF 8 FOOD ODORANTS AND 2 BLANKS

Table (35). Results of the odor identification study (percent correct response) between the elderly and college students, Experiment II.

ODOR RECOGNITION-EXPERIMENT II,[1,2]

Foods	College[3]		Elderly[4]	
	A	B	A	B
Grape	90	97	22	29
Tuna fish	71	90	21	25
Tomato paste	58	87	11	25
Blank 1	86	97	17	30
Lemon	86	100	39	42
Pepper	79	100	29	21
Maple syrup	44	70	18	21
Blank 2	80	94	21	33
Apple juice	72	70	17	33
Garlic powder	87	93	26	21
$\bar{x}$ =	75	90	22	30
S.D. =	15	11	8	8
χ^2 =	25.6***	12.6***	22.4***	17.2***

[1]A: Trial A without a list of the food odorants.

[2]B: Trial B with a list of the food odorants.

[3]: t-test A vs. B = t = -4.81***

[4]: t-test A vs. B = t = 3.79**

Table (36). Names of food odors incorrectly given to the blank samples #1 and #2 by the college students of Experiment II.A, B.

ODOR RECOGNITION (WITHOUT LIST) EXPERIMENT II, A.	
Blank #1	Blank #2
tuna (3)[1]	grape (2)
beef gravy	maple syrup
bacon fat	raisins
cooking grease	apple juice
vanilla	fruity
peaches	(preceded by maple syrup)
(preceded by tomato paste)[2]	
ODOR RECOGNITION (WITH A LIST) II.B.	
Blank #1	Blank #2
peaches	apple juice
(preceded by garlic powder)	cranberry juice (2)
	(preceded by tomato paste)

[1]Number in parentheses means number of subjects who made the same guess.

[2]The blank sample was preceded by the garlic food odorant.

Table (37). Names of food odors mistakenly given to the blank samples #1 and #2 by the elderly subjects of Experiment II. A, B.[1,2]

ODOR RECOGNITION (WITHOUT LIST) II.A.	
Blank #1	Blank #2
fruit juice	fruity
bread	pickles
orange	

ODOR RECOGNITION (WITH A LIST) II.B.	
Blank #1	Blank #2
grape juice (3)	lemon
peach (2)	
apple juice (3)	
tuna fish	

[1]Order of food odorants to the elderly identical to the college students.

A correlation coefficient of the age versus the total scores for each elderly person is tabulated in Table (38). Although a negative relationship does exist between age and the ability to recognize odorants, the correlation is not strong.

Figures 15 and 16 depict a comparison of the odor identification scores of the 15 male and 15 female college students in Experiment II. The rationale behind this dichotomy of gender was to determine if female participants were more sensitive and aware of food odorants than males. Inspection of both figures, however, finds the male students slightly better at recognition with and without a list, although the two scores were very close (see Table 39).

Table (38). Correlation of odor recognition scores (out of 21 possible) and age, in the elderly of Experiment II.A. (without) and II.B. (with a list).[1]

A: Range of scores Experiment II.A. (0-14)

B: Range of scores Experiment II.B. (0-18)

C: Range of ages: 74-95 years.

	A	B	C
A	1	.57** (.32)	-.37 (.14)
B		1	-.41 (.17)
C			1

1. A vs. C: y= 37 - .39 (x)
2. B vs. C: y= 54.95 - .58 (x)
3. A vs. B: y= 2.48 + .42 (x)

[1]Values in parentheses are (R^2)

**, Significant at P<0.01.

Figure 15. Comparison of the odor identification scores (without a list) between the male and female college students of Experiment II.A.

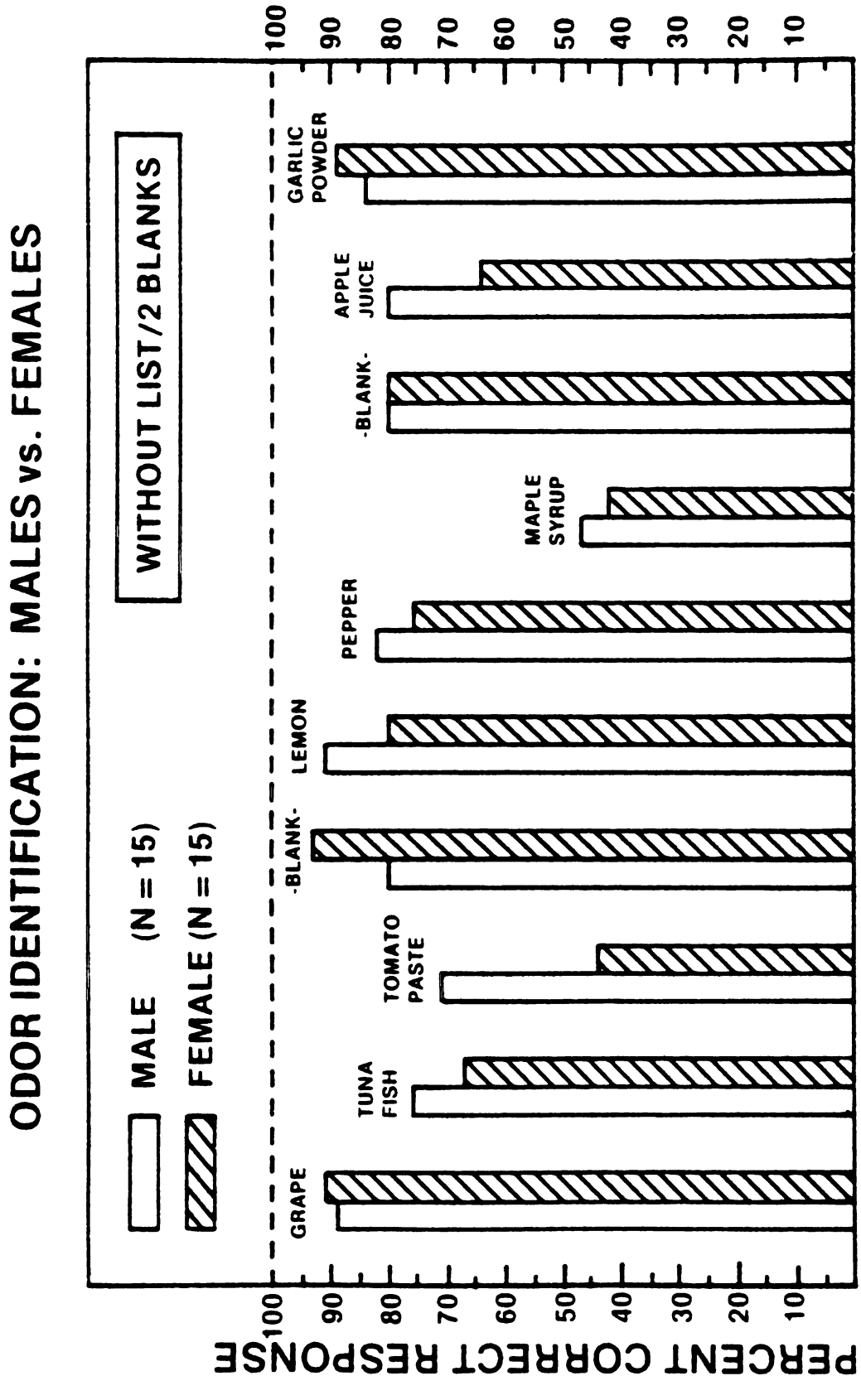
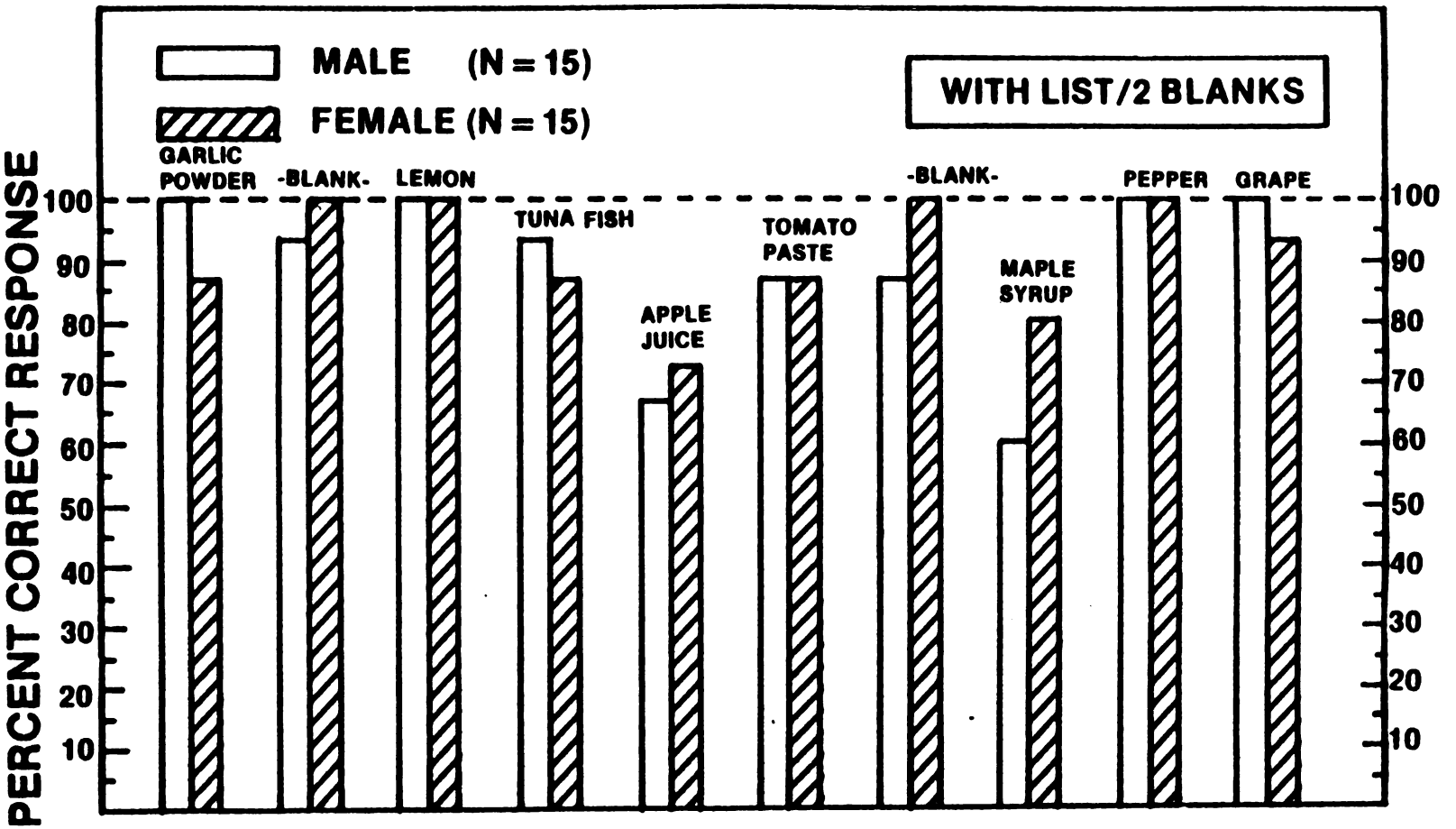

ODOR IDENTIFICATION: MALES vs. FEMALES
MALE (N = 15)
FEMALE (N = 15)
WITHOUT LIST/2 BLANKS
PERCENT CORRECT RESPONSE
100
90
80
70
60
50
40
30
20
10
GRAPE
TUNA FISH
TOMATO PASTE
-BLANK-
LEMON
PEPPER
MAPLE SYRUP
-BLANK-
APPLE JUICE
GARLIC POWDER
AVERAGE SCORE OF 8 FOOD ODORANTS AND 2 BLANKS

Figure 16. Comparison of the odor identification scores with a list, between the male and female college students of Experiment II.B.

ODOR IDENTIFICATION: MALES vs. FEMALES



AVERAGE SCORE OF 8 FOOD ODORANTS
AND 2 BLANKS

Table (39). Comparison of the combined odor identification scores (both sessions A,B) between the male and female college students in Experiment II.A, B.

Food Odor	Male	Female
Grape	94 (%)	92 (%)
Tuna fish	84	77
Tomato paste	79	66
Lemons	96	90
Pepper	91	88
Maple syrup	53	61
Apple juice	73	69
Garlic powder	92	88
	$\bar{x}$: 82.8	78.9

[1]Total possible points: 90(3 points x session A,B, with 15 subjects), i.e. 85/95 x 100 for grape equals 94%.

[2]Student's t-test for paired variates: $t=1.86^{ns}$

C. Experiment III. Zinc/Copper Supplementation of Eight Elderly Subjects.

Subjects

The anthropometric particulars of the eight elderly volunteers are listed in the Appendix, Table (21B). The average age of the seven women, one man group was 82.5 years; average height was 159.5 cm and the average weight was 62 kilograms. The body weights for the entire group over eight weeks changed very slightly--62 kg before the supplementation and 61.5 kg after.

Part 1. Odor recognition with and without a list.

At the completion of Experiment II, eight subjects volunteered to take the zinc/copper supplement for eight weeks. Subject data labeled "before zinc/copper" were collected when subjects were participants of Experiment II. Data and graphics labeled "after zinc/copper" were collected immediately after the eight week supplementation regimen.

The results of the odor identification experiment without a list is illustrated by Figures 17 and 18. Although the graphics show an increase in recognition for orange and grape, the overall average is very similar, as if a replication were made. Table (40) contains the means and statistics of the elderly recognition data after zinc/copper supplementation. In both instances, before and after supplementation, the data were not significantly different.

Figure 17. Odor identification by the eight elderly to 10 food odorants, before and after the Zn/Cu supplementation of Experiment III.1, without a list.

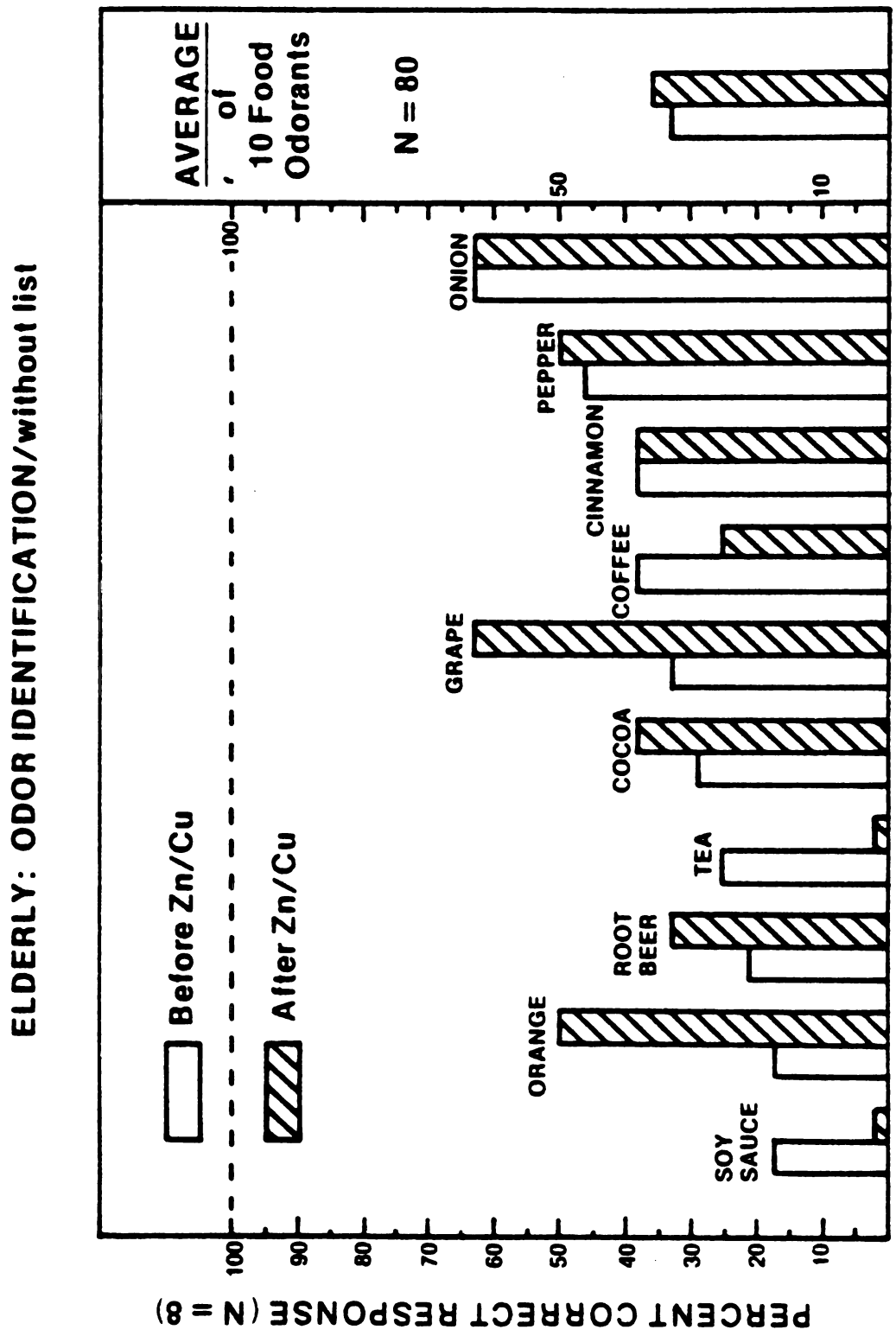

ELDERLY: ODOR IDENTIFICATION/without list
Before Zn/Cu
After Zn/Cu
AVERAGE of 10 Food Odorants
N = 80
PERCENT CORRECT RESPONSE (N = 8)
100
90
80
70
60
50
40
30
20
10
50
10
ONION
PEPPER
CINNAMON
COFFEE
GRAPE
COCOA
TEA
ROOT BEER
ORANGE
SOY SAUCE
TEN FOOD ODORANTS

Figure 18. Odor identification by the eight elderly to 10 food odorants, before and after the zinc/copper supplementation of Experiment III.1, with list.

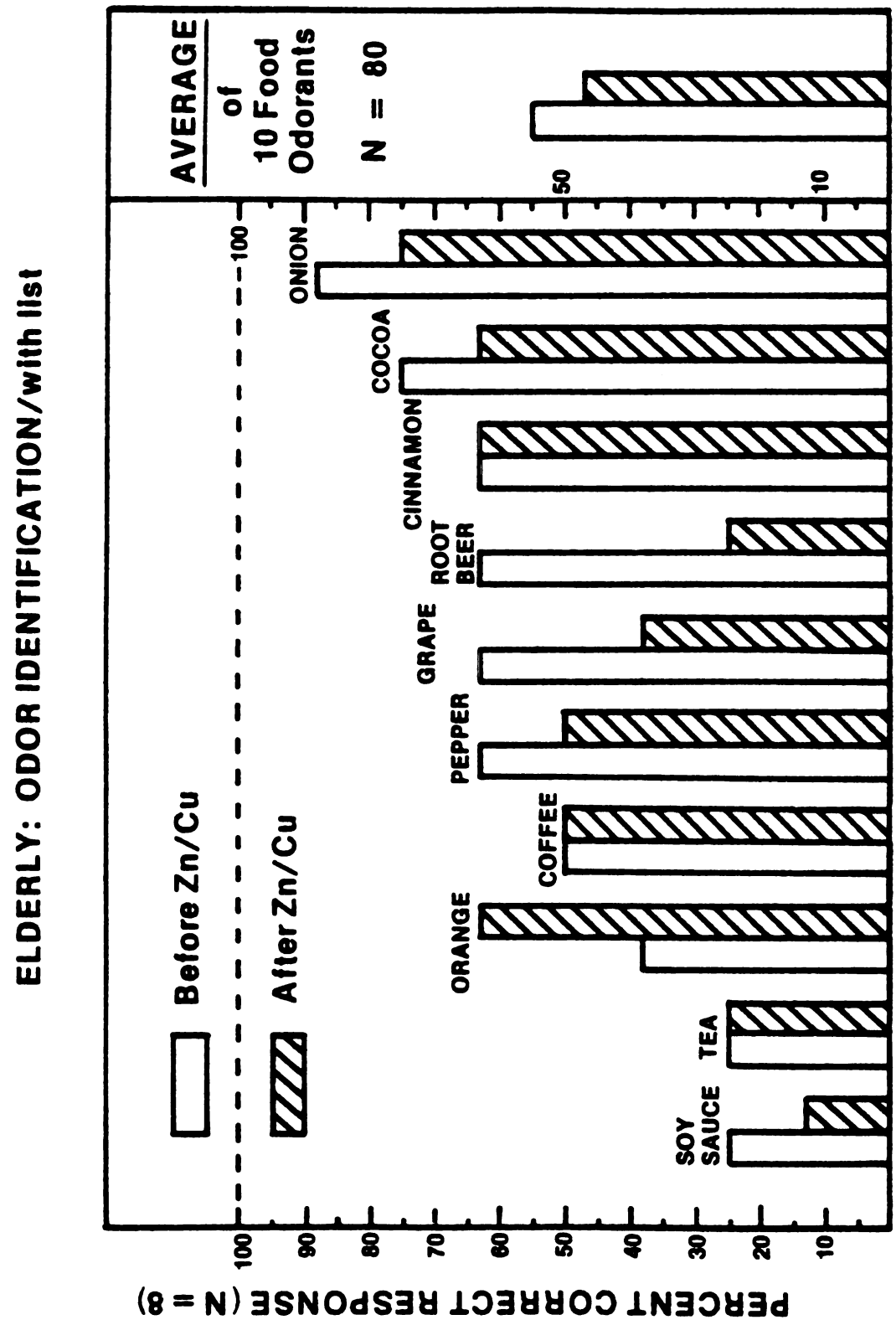

ELDERLY: ODOR IDENTIFICATION/with list
Before Zn/Cu
After Zn/Cu
AVERAGE
of
10 Food
Odorants
N = 80
PERCENT CORRECT RESPONSE (N = 8)
TEN LIST-GIVEN ODORANTS
ONION
COCOA
CINNAMON
ROOT BEER
GRAPE
PEPPER
COFFEE
ORANGE
TEA
SOY SAUCE

Table (40). Means of the correct percent response to the odor identification of Experiment III.1, with and without a list, in eight elderly subjects, before and after supplementation with Zn/Cu.

	A		B	
	Without a List[1]		With a List[2]	
Food	Before[3]	After	Before	After
Soy Sauce	17	0	25	13
Pepper	46	50	63	50
Grape	33	63	63	38
Cocoa	29	38	75	63
Tea	25	0	25	25
Root Beer	21	33	63	25
Coffee	38	25	50	50
Cinnamon	38	38	63	63
Orange	17	50	38	63
Onion	63	63	88	75
$\bar{x}$: (mean)	33	36	55	47

[1]Student's t-test: -0.55 (not significant)

[2]Student's t-test: 1.65 (not significant)

[3]Before supplementation of Zn/Cu

Part 2. Odor intensities

The similarities of odor recognition carried over to the odor intensities. Figure 19 illustrates that the average intensities of seven food odorants were quite similar, before and after Zn/Cu supplementation. Because the trend for five of the seven foods was for increased odor intensity, the four-way AOV found the difference significant at the $P<0.01$ level (Table 41). If one pools the non-significant 3-way interactions, as some statisticians suggest, the before/after F-ratio is not significant. In any event, the trend for most of the food odors was increased odor intensity after the mineral supplementation. The group by odorant interaction (AXB) was also significant, indicating that the Zn/Cu supplementation affected the way the panelists rated all the odorants. However, this interaction was low ($P<0.05$) and would be non-significant in pooled data.

Part 3. Salivation to food stimuli

Figure 20 represents the effect of Zn/Cu on whole-mouth salivation in the eight elderly. Although the two resting levels and tasting lemonade were comparable, there existed an unexpected drop in the response to sniffing lemons after the mineral tablets. Examination of the AOV (Table 42) reveals significant overall means before and after supplementation, highly significant experimental sequence and subject variability, and a non-significant replication factor. The group by sequence interaction was significant at the $P<0.05$ level. Inspection of the individual data revealed that one subject nearly doubled her overall salivation rate to food stimuli by participation

in the eight week supplementation regimen. Interestingly, the subject mentioned previously to the experimenter that her salivation had increased dramatically to food stimuli or no stimuli at all.

Throughout the zinc/copper supplementation period, a log was kept each week concerning the subjective remarks by the elderly to the effect(s) of supplementation on taste and aroma perception. Table (25) in the Appendix catalogues these remarks as the study progressed through the eight week period. Although few comments were made concerning changes in gustatory or olfactory acuity, subject number 6 mentioned an increase in salivation near the completion of the experiment.

The A X C interaction (before/after by subject) was highly significant, as three subjects increased their overall secretory rates and five decreased. A cautious interpretation of the data suggests that the observed effects were more random than the statistics indicate, especially in non-pooled AOV's of significant variability.

Part 4. Hair Zinc/Copper analyses

The zinc/copper analyses of the hair samples from the elderly subjects before and after zinc/copper supplementation is listed in Table (43). After the eight week supplementation program, hair zinc values increased from 210 ug/g to 231 ug/g of hair. This increase, although not statistically significant at $P<.05$, was, nevertheless, of considerable importance as a trend for significance was noted at $P<.10$. Copper levels, however, demonstrated a slight, nonsignificant increase at 28 ug/g to 33 ug/g of hair. It should be pointed out that the variance between subjects was considerable.

Figure 19. Odor intensity by the eight elderly to seven food odorants, before and after the zinc/copper supplementation of Experiment III.2.

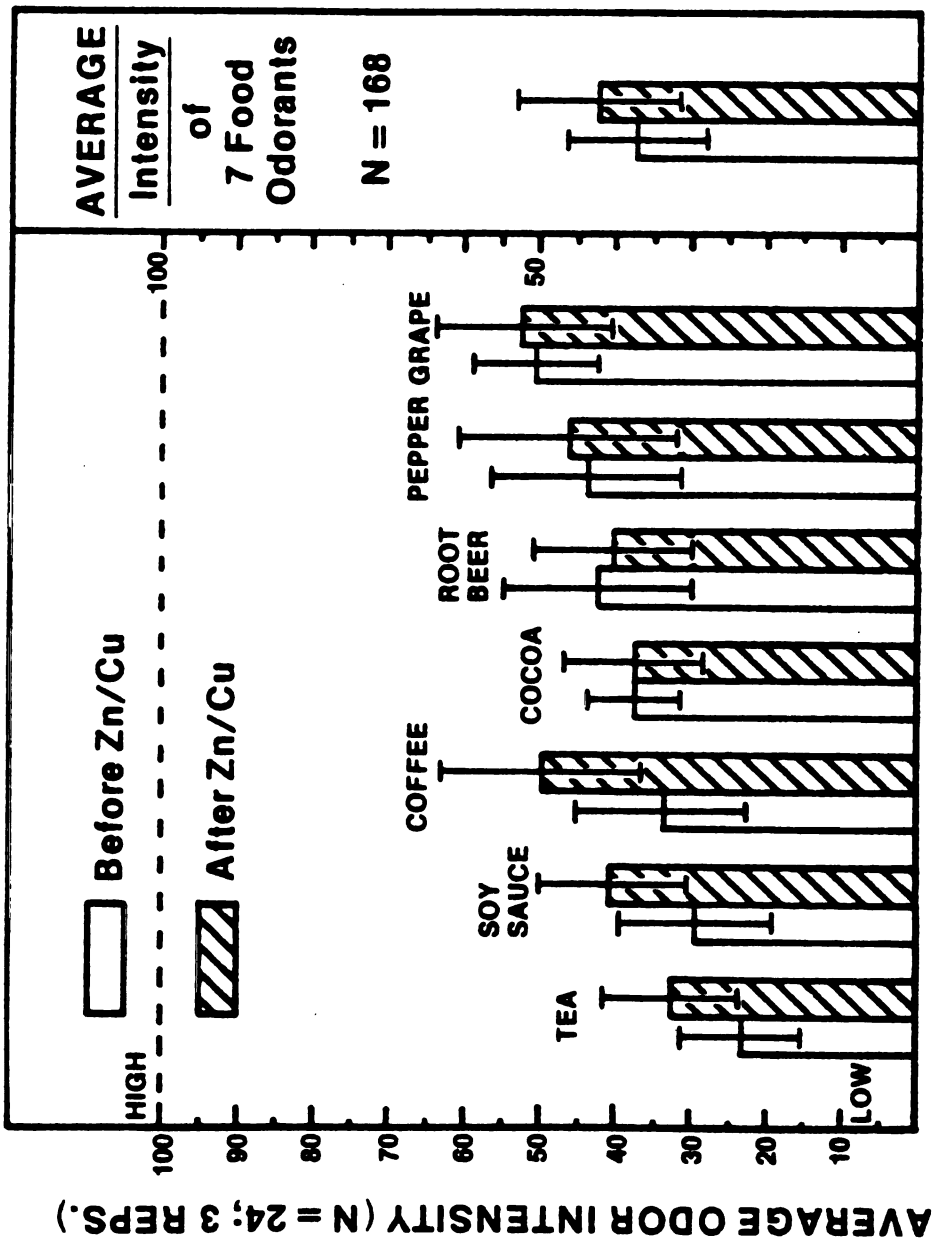

ELDERLY: ODOR INTENSITY
Before Zn/Cu
After Zn/Cu
AVERAGE
Intensity
of
7 Food
Odorants
N = 168
HIGH
LOW
100
90
80
70
60
50
40
30
20
10
TEA
SOY SAUCE
COFFEE
COCOA
ROOT BEER
PEPPER GRAPE
AVERAGE ODOR INTENSITY (N = 24; 3 REPS.)
SEVEN OLFACTORY STIMULI

Table (41). Four-way AOV of the effect of Zn/Cu (before and after) on the odor intensity of seven food odorants, Experiment III.2.

Source of Variation	D.F.	S.S.	M.S.	F-ratio
A- Before/after Zn/Cu	1	2552.13	2552.13	11.77**
B- Sequence	6	16015.4	2669.23	12.31***
C- Subjects	7	87571.4	12510.2	57.69***
D- Replications	2	2050.25	1025.12	4.73*
A X B	6	3210.38	535.06	2.47*
A X C	7	13839.7	1977.11	9.12***
B X C	42	22126.6	526.82	2.43*
A X D	2	934.13	467.06	2.15
B X D	12	2951.	245.92	1.13
C X D	14	3517.38	251.24	1.16
A X B X C	42	11499.	273.79	1.26
A X B X D	12	3234.75	269.56	1.24
A X C X D	14	8371.	597.93	2.76**
B X C X D	84	17750.	211.31	0.97
A X B X C X D	84	18214.9	216.84	

Note: Error term (A X B X C X D) used as denominator for all F-ratio calculations.

*, **, ***, Significant at P<0.05, 0.01, and 0.001, respectively.

Figure 20. Whole-mouth salivation by the eight elderly to sniffing and tasting lemonade, before and after the zinc/copper supplementation of Experiment III.

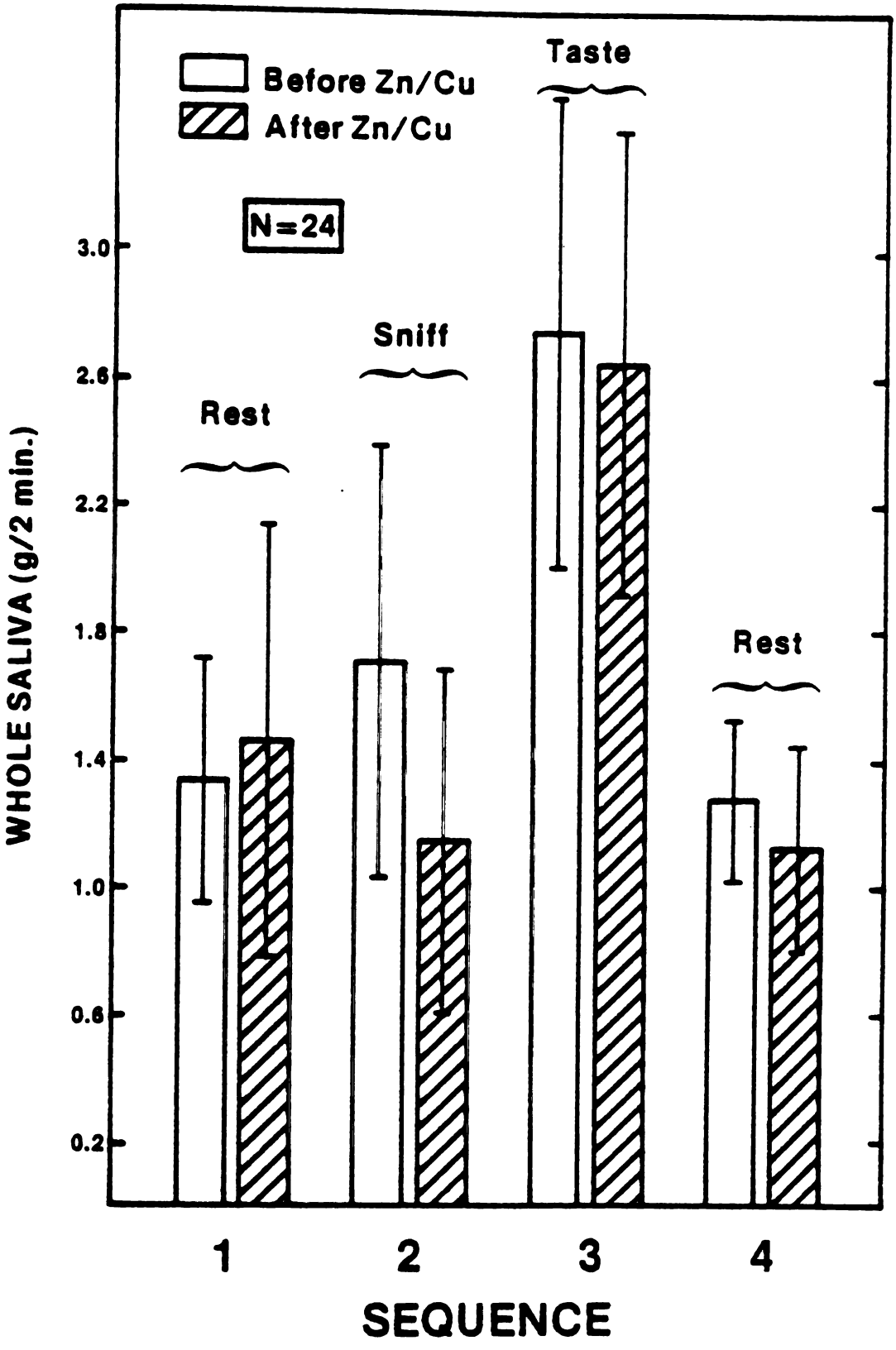

Before Zn/Cu
After Zn/Cu
N=24
Rest
Sniff
Taste
Rest
3.0
2.6
2.2
1.8
1.4
1.0
0.6
0.2
WHOLE SALIVA (g/2 min.)
1
2
3
4
SEQUENCE

Table (42). Four-way AOV of the salivation levels to sniffing and tasting lemons and lemonade by eight elderly, before and after Zn/Cu supplementation, Experiment III.C.

Source of Variation	D.F.	S.S.	M.S.	F-ratio
A- Before/after Zn/Cu	1	1.45	1.45	4.68*
B- Sequence	3	67.78	22.59	72.87***
C- Subjects	7	152.69	21.81	70.35***
D- Replications	2	0.36	0.18	0.58
A X B	3	2.93	0.98	3.16*
A X C	7	31.04	4.43	14.29***
B X C	21	22.49	1.07	3.45***
A X D	2	0.61	0.30	0.97
B X D	6	1.28	0.21	0.68
C X D	14	15.31	1.09	3.52***
A X B X C	21	10.21	0.49	1.58
A X B X D	6	2.49	0.41	1.32
A X C X D	14	11.92	0.85	2.74**
B X C X D	42	11.65	0.28	0.90
A X B X C X D	42	12.85		

Note: All F-ratios used the error term.

*, **, ***, Significant at P<0.05, 0.01 and 0.001, respectively.

Table (43). Zinc/copper analyses of hair samples from the elderly subjects, before and after zinc/copper supplementation in Experiment III.

Subject	Before/After (Zinc)[2]	Before/After (Copper)[3]
8	330/400 ug/g[1]	61/43
11	113/137	19/23
12	193/240	27/33
13	214/210	33/57
17	241/235	19/24
18	214/240	30/30
23	214/201	17/19
25	161/191	14/31
	210±63/223±76	28±15/33±12

[1]All values in micrograms/gram hair sample.

[2]t-test: before/after zinc =-2.16 (not significant)

[3]t-test: before/after copper = -1.14 (not significant)

REFERENCES

1. Hewlett-Packard. "Statistics manual for use with the HP-55", 1974.

2. Keppel, G. "Design and analysis, a researcher's handbook". Prentice-Hall, New Jersey, 1973.

3. Roessler, E.B. "Applied statistics in the agricultural sciences". Syllabus for Ag Stats. 150, UC Davis, September, 1975.

4. Schiffman, S. Food recognition by the elderly. J. Gerontology, 32, 586-592, 1977.

V. DISCUSSION

A. STUDE I. Sensory Scaling, Salivary Flow Rates and Odor Recognition between Twenty-Five College Students and Twenty-Five Elderly Subjects.

Sensory Scaling

In the analysis of the hedonic responses to varied levels of NaCl in tomato juice or to the sucrose levels in lemonade, one must remember the great variability of response inherent in hedonic judgments. As was pointed out by Trant and Pangborn (1981), a significant statistical difference in hedonic scores may obscure the correct interpretation of individual variance. In the tomato juice experiment, although the elderly and college students judged the overall degree of liking for the samples as being significantly different, inspection of the individual graphics reveals an amazing similarity in response. While elderly possessed a slightly restricted range of response, only a few individuals had difficulty in the sensory discrimination of the samples. A similar situation existed for the lemonade hedonic scores--the two groups were significantly different in overall hedonic scores but inspection of the individual graphics revealed remarkable similarity in direction and magnitude. Similarly, truncation of the intensity responses to NaCl levels in tomato juice and sucrose in lemonade was noted in the elderly. Inspection of the individual data, however, found that two subjects possessed flat sensory functions for both the hedonic and intensity

scalings. In the only other study on age and suprathreshold sensory scaling, Enns et al. (1979) concluded that old age does not produce a noticeable deficit in suprathreshold sensory scaling. It must be pointed out, however, that the mean age of their subject population was 71 years as compared with the 83 years of age in Experiment I.

The failure to find moderate gustatory deficits in suprathreshold scaling could not have been predicted with the literature on discrimination of threshold testing. A great majority of the sensory studies found taste impairment in the elderly when threshold measurements were taken (Hinchcliff, 1958; Richter and Campbell, 1940; Cooper et al., 1959; Hermel et al., 1970; Balogh and Lelkes, 1961; Cohen and Gitman, 1959; Greger and Geissler, 1978; Langan and Yearick, 1976). Grzegorczyk et al. (1979), however, criticized many of these early threshold studies by virtue of their poor methodologies, inadequate subject numbers, or contradictory conclusions. Grzegorczyk et al., in their threshold data, concluded that the elderly possess only a slight impairment in threshold abilities.

In the light of the recent study by Grzegorczyk et al. (1979) and the suprathreshold study by Enns et al. (1979), one may conclude that while the elderly may have a slight to moderate impairment in absolute or detection thresholds to one or more of the four basic tastes, suprathreshold measurements are less affected than one would predict from the myriad of literature threshold studies.

Olfaction

In contrast to the sensory studies on gustation which demonstrated a remarkable similarity of response between the elderly and college students, the results of the olfactory studies--where the elderly, in general, averaged a percent correct response one-third that of the college students--were quite unexpected. In fact, every effort was made to placate the concerned panel members when they were unable to detect the aroma in any of the ten samples. Quite often the panelists commented on their lack of olfactory acuity with such remarks as "my sniffer isn't working today", and "I haven't been able to smell (foods) for a number of years now".

A review of the individual aroma identification revealed that about one-half of the elderly (12/25) possess essentially no ability to detect food aromas as presented. Even foods with a high degree of volatility (eg., onions or pepper), failed to elicit an increase in recognition. Although many of the panelists did not identify the onion and pepper samples, a few subjects did comment on a tingling sensation present in the nasal cavity. It was as if the "quality" of the odorant was absent, while the trigeminal or "pain" component continued to respond, however weak.

Although olfactory sensitivity testing has not received as much research as gustatory discrimination, the few studies that were completed demonstrate a surprising concordance with the present study. Chalke and Dewhurt (1957), Kimbrell and Furchtgott (1963) and Venstrom and Amoore (1968) all agree that olfactory acuity in the aged is decreased compared to younger age controls (subjects generally under 40 years of age). Schiffman (1979) in a study on olfactory sensitivity in the

elderly demonstrated that the elderly (mean age 73.3 years) had moderate difficulty in identifying five fruit aromas; i.e., cherry, grape, lemon, orange and tomato. In comparing Schiffman's study with the present work, it must be noted that her subjects tasted pureed food samples while blindfolded, which contrasts with the straight olfactory testing here. The additional recognition cue of tasting the pureed food complicates a comparison of the data. Therefore, it is not surprising that Schiffman's subjects were able to identify the foods with a greater degree of accuracy than in Experiment I.

Schiffman (1979), in the discussion on olfaction and aging, speculated that memory impairment may be a significant factor in the decreased recognition scores for food odorants. The design and outcome of Experiment I.3, part 2, however, must cast some doubt on this conclusion. The addition of a "list" of the odorants in the sensory testing booth increased the accuracy of the elderly's response only 20% over the previous level.

An intriguing question arose during the intensity testing of the seven food odorants by the elderly. Is it possible to perceive an odorant without being able to identify it? The data of Experiment I suggests an affirmative answer. As mentioned previously, 12/25 subjects possessed essentially no ability to recognize food odorants; however, during the food aroma intensity rating sessions, these same subjects perceived a food aroma and rated it as such. Although this experimenter cautioned the subjects to give a zero value to aromas not perceived, subjects consistently gave intensity ratings to foods they could not identify. Although there is no precedent in the literature demonstrating this effect, one might speculate a

presence of a "universal" aroma that might be present and perceived. This nondescript aroma might arise from a failure to integrate lower order intensity perceptions. Schiffman (1979), in a review on the biological aspects of olfaction and aging, suggested that there are age-related structural degradations in the hippocampus and amygdala. If these two areas in the brain are responsible for quality interpretation, then the possibility of perceiving a stimulus without discrimination is a plausible conclusion. An alternate hypothesis might be that the surface cilia in the olfactory region, through the aging process, have increased detection thresholds to a point where sensory input does not support sensory discrimination.

Schiffman (1979) concluded that:

> ". . . the loss of taste quality information, i.e., loss of distinctions between stimuli, following neural losses means that all stimuli must taste somewhat more similar to each other than before such losses. In other words, with neural losses various stimuli may tend to elicit a common . . ."

By substituting the word "taste" with "olfaction", the same conclusion must be drawn here.

Salivation

One way to check the validity of the olfactory intensity data by the elderly might be to measure salivation induced by the aroma of foodstuffs. Reflex salivation is known to be a fairly linear process--higher strengths of odorants induce greater salivary secretions (Pangborn et al. 1979). Therefore, one might speculate that the elderly would not salivate as greatly to the aroma of the lemons as the college students by virtue of their poorer intensity responses to the seven

food aromas. The results indicate that the elderly, in general, did not salivate across all treatments to the same level as the college students. In particular, responses to sniffing the lemons and tasting lemonade were depressed moderately. This decrease in salivation in the elderly group agrees with the few published reports in the literature (Meyer et al., 1937; Wainwright, 1943; Gutman and Ben Aryeh, 1974; and Mäkila, 1977).

Although not stated explicitly in his article, Chauncey et al. (1980) found little correlation between age and salivary flow rates. The population tested, however, was divided into two groups--above and below 55 years of age. Only 10 of his subjects were above 60 years of age, which may be an insufficient number to delineate differences in salivation when the total group tested was 153 individuals.

Nutrient Analysis

Calculation of nutrient intakes from the 3-day dietary recalls revealed surprisingly little differences between the elderly and college age subjects. Only total kilo-calories and magnesium levels were found to be lower in the elderly than in the university students. This result is not surprising, considering the fact that the elderly resided in a retirement facility with an excellent food service program. Using the 1974 RDA as a comparison, the college students were somewhat low in kilo-calories, pyridoxine, folacin, vitamin D, copper and zinc. Inspection of the individual dietary records revealed the abnormally low levels of kilo-calories among the thirteen women participants. Eight of the women recorded total calories less than 1200 kcals. The

current fashion trend to stay slim through dieting may be responsible for these results. Indeed, White (1976) mentions that diets low in energy values are not unusual for college women. Diets low in kilocalories usually are low in other nutrients as well (White, 1969), especially the mineral elements. Therefore, it is not unusual, when all the data from the males and females are averaged together, to find the diets low in certain mineral levels such as zinc and copper. As White (1976) and Holden et al. (1979) point out, caloric intakes less than 1000 kcals rarely provide 100% of the RDA for zinc or copper. The data on the college students, however, agree with the report by Holden et al. (1979) which found 4.19 mg zn/1000 kcals as compared with 4.5 mg zn/1000 calories in our study.

Surprisingly, the elderly, although below the RDA's for pyridoxine, folacin and vitamin D, were a bit higher in their levels of zinc and copper (1.884 and 10.35 mg, respectively) than university students. These values are similar to the values of Deeming and Weber (1978)--11.5 mg zn and 1.7 mg cu--and Marshall et al. (1975).

Hair Analysis

In Study I, the university students averaged 214 mcg of zinc and 39.2 mcg of copper per gram of hair sample, while the elderly's values were somewhat lower with 188.3 mcg of zinc and 27.5 mcg copper per gram of hair. In the discussion of zn/cu levels in hair and the comparison of the two age groups, it is useful to refer back to Table 9 of the dissertation. Generally speaking, the values for zn/cu per gram of hair sample in the university students were a bit higher than most

of the studies cited, but well within the range of variance noted. A recent report by Vir and Love (1981) reconfirms this finding. In a study of 24 young women comparing the effects of oral contraceptives on zinc and copper nutriture, the authors found an average zinc content of hair near 175 μg/g and of copper, 13.4 μg/g in the control subjects. Large standard deviations in these studies underscore the need to be cautious in absolute number comparisons.

Fewer studies have determined the trace mineral contents of zinc/copper in the elderly. Vir and Love (1979) studied the zinc and copper status of the elderly with ages ranging from 65 to 95 years. These subjects were studied under two groups, with and without multivitamin supplementation. The control groups showed a mean zinc value of 208 μg/g hair and a mean copper level of 12.0 μg/g hair. It is interesting to note that the range of zinc values for the elderly group was 85-490 μg zn/g hair. In the multivitamin group (no zinc or copper added) mean zinc values increased to 245 μg/g hair and copper to 15.8 μg/g hair. The apparent association between the increase in vitamin intake and plasma or hair level of cations was not explained by the authors. However, it does serve as a caveat to investigators on the variety of factors that might influence trace metal nutriture.

As far as the influence of age on zinc and copper contents of hair is concerned, the literature is markedly equivocal. Kelvay has stated that both copper and zinc levels in hair increase with the age of the subject (Klevay, 1970a; Klevay, 1970b). Deeming and Weber (1978), however, stated that not only do hair minerals correlate poorly with serum levels or dietary intake, hair zinc decreased with age. Petering (1971), however, demonstrated that female subjects increase

their hair copper with age but hair zinc decreases. Males, on the other hand, show a decrease in both hair zinc and copper with age. To further confuse the issue, Hambidge (1973) emphatically states that interpretation of analytical data on hair copper concentrations requires great caution. Analyses, the author states, should be limited to recently grown hair within 1 to 2 cm of the scalp.

It is obvious that until a more satisfactory method of measuring trace elements in hair appears (e.g., erythrocytic zinc, Robson and Spell, 1981), the researcher must exercise extreme caution in the preparation of samples and the interpretation of data in all hair biopsy studies.

B. EXPERIMENT II. Odor Identifications in the Elderly and College Students.

The research intentions of Experiment II were twofold. First, to discern whether the elderly subjects would improve their odor recognition scores with practice or with a list of foods placed in the booth; and secondly, to enumerate what classes of foods, if any, the elderly might assign to the blank sampling cups. In Experiment II, five new foods were added to the list, two of the odorants were retained from the first experiment and the two blank cups were randomly placed with the others.

As in Experiment I, the elderly experienced great difficulty in odor recognition, even with the list of foods appearing in the sensory booth. The two food items which the elderly first encountered in Experiment I received similarly poor recognition scores. If experience were a factor in aroma identification, one might expect a higher correct response than the results indicated. The college students, in marked contrast, were able to approach 100% recognition for all but the difficult-to-identify maple syrup. Of considerable interest was the finding that the elderly were able to identify the blank sample 50% of the time. This result suggests that the elderly, while having great difficulty with aroma discrimination, could still identify an empty sample cup. Again we see support for the theoretical "universal" aroma of the elderly--a phenomenon where the quality of an aroma is not discernable but the perception of an undefinable stimulus is. Although Schiffman (1977) commented in her paper on food recognition and the elderly that the decline in smell sensitivity is greater

than the decline in taste sensitivity, the presence of a universal aroma was not postulated as an explanation for sensory decline.

The grouping of misnamed food aromas by the elderly did not form any discernible trend or classification. Curiously, Schiffman (1979) found that elderly subjects were better at guessing fruit aromas over any other categories, i.e. meat, milk, vegetables, etc. In Experiment II, the elderly were encouraged to verbalize all guessing; however, the natural tendency of these subjects to be conservative in their opinions may have masked the proper interpretation of our results.

C. EXPERIMENT III. Zinc/Copper Supplementation of Eight Elderly Subjects.

Although several dozen studies in the literature examined the use of zinc/copper to restore or treat taste and smell dysfunction, (Henkin, et al., 1969), only one study attempted to improve taste acuity in the elderly. In this study, Greger and Geissler (1978) found that 15 mg of zinc for 95 days essentially unaffected the taste acuity thresholds for the four basic tastes. In our study, although copper was added and the zinc dosage was higher, the findings echo those of the 1978 study by Greger and Geissler. Odor recognition, aroma intensities and salivation to food stimuli were essentially unchanged. In fact, some of the data on odor identification and intensity suggest a decrease in response after the zinc/copper supplementation. These artifacts of statistical data do little except confuse the interpretation of the supplementation results. The salivation data are of even greater variability. Although the decrease in salivation to food stimuli is not significantly different, this curious response remains unexplained at present.

Several possible explanations might exist to explain the lack of response to zinc/copper therapy. First, and most obvious, is the confusion surrounding the link between trace metal ingestion and sensory restoration (Catalanotto, 1978). This author concluded that zinc depletion can lead to decreased taste acuity, but decreased taste acuity is not necessarily associated with depletion of zinc. Since both the dietary recalls and hair mineral analysis of the elderly in Experiment I do not suggest a depleted zinc/copper body state, it is not

surprising that additional Zn/Cu in the diets failed to affect sensory acuity.

A second explanation concerns that actual amount of Zn/Cu absorbed from the supplement by the elderly. The hair analyses of the elderly after supplementation demonstrated only a moderate increase in Zn/Cu after eight weeks of mineral ingestion. Perhaps the levels of added Zn/Cu were insufficient to increase body stores to a level where sensory acuity is affected. Aamodt et al. (1981) demonstrated that human zinc absorption is age dependent. In their study, 75 volunteers aged 18-84 were studied for zinc absorption using total body counting of Zn 65. The authors concluded that zinc absorption decreases linearly with age in both men and women. In another study, Taper et al. (1980) added supplements and $ZnSO_4$ and $CuSO_4$ to a three day cycle menu in ten subjects aged 68.1 years. The authors noted a great variability in the retention of zinc in their patients, with the level of zinc intake (7.8 or 23.3 mg/day) having no effect on zinc retention. Both of the studies cited above demonstrate the difficulty in increasing body stores of Zn/Cu, whether used in dietary intake or absorption studies. In fact, Friedman et al. (1980) found that hair zinc and salivary sediment zinc levels did not increase after 15, 50 and 100 mg zinc. Perhaps the form of the supplement used is of greater importance than the amount. In any event, additional research on Zn/Cu absorption and retention is needed to discern the most efficient method of increasing the mineral stores of the body.

VI. GENERAL CONCLUSIONS

Gustation

The overall results of the taste responses to sucrose in lemonade and salt in tomato juice suggested a great similarity in response between the college students and the elderly. Although there was evidence of a flattened psychophysical function in the overall response, the suprathreshold responses were not nearly as depressed as one would expect from a prior knowledge of the threshold studies in the literature.

Olfaction

Quite unanticipated were the results of the olfactory studies demonstrating a highly depressed level of olfactory acuity and recognition exhibited by the elderly. Although the literature contains several reports of decreased olfaction in older subjects, the extent of the decline was hardly anticipated. The anecdotal accounts of the little old lady who applies too much perfume in grooming for church activities is now partially explained. As to the etiology of this condition of anosmia, whether partial or complete, one must wait further investigation. Why olfaction is more affected than gustation remains another question subject to future scientific inquiry. While Schiffman (1980) has speculated on the finding of atrophy in certain brain structures relating to olfaction, why the sense of taste is not as profoundly affected remains unclear.

Zinc/Copper and Taste

Although the attempt to restore "normal" olfactory acuity in the elderly with the addition of Zn/Cu to the diets was unsuccessful, the results, however negative, shed light on the complexity of linking mineral nutrition with a psychophysical task. Aging is a phenomenon of complex organ and subcellular changes over time. The likelihood of reversing any one of these changes with a simple change of diet must be considered a formidable task at best. Simple solutions, while therapeutically and experimentally enticing, have yet to be elucidated by this or anyone else's research protocols. Perhaps the etiology of decreased sensory acuity with age is a simple one. The application of this knowledge, however, to reverse or delay this impending decline is likely to be a challenging task, not only for psychophysicists involved in its resolution, but also medical personnel involved with its application.

REFERENCES

1. Aamodt, R.L., Rumble, W.F., Johnston, G.J., Markley, E.J. and Henkin, R.I. Human zinc absorption is age dependent. Fed. Proc., 40, 950, 1981.

2. Balogh, K. and Lelkes, K. The tongue in old age. Gerontol. Clin., 3, 38-54, 1961.

3. Catalanotto, F.A. The trace metal zinc and taste. J. Am. Clin. Nutr., 31, 1098-1103, 1978.

4. Chalke, H.D. and Dewhurst, J.R. Coal gas poisoning: loss of sense of smell as a possible contributory factor with old people. Br. Med. J., 2, 1915-1917, 1957.

5. Chauncey, H.H., Wallace, S. and Alman, J.E. Salivary Chloride Levels, Taste Thresholds for Salt, and Food Ingestion. In: "Biological and Behavioral Aspects of Salt Intake", Academic Press, pp. 113-125, 1980.

6. Cooper, R.M., Bilash, I. and Zubek, J.P. The effect of age on taste sensitivity. J. Gerontol., 14, 294-298, 1959.

7. Deeming, S.B. and Weber, C.W. Hair analysis of trace minerals in human subjects as influenced by age, sex, and contraceptive drugs. J. Am. Clin. Nutr., 31, 1175-1180, 1978.

8. Enns, M.P., Van Itallie, T.B. and Grinker, J.A. Contributions of age, sex and degree of fatness on preference and magnitude estimations for sucrose in humans. Physiol. Behav., 22, 999-1003, 1979.

9. Greger, J.L. and Geissler, A.H. Effect of zinc supplementation on taste acuity of the aged. J. Am. Clin. Nutr., 31, 633-637, 1978.

10. Grzegorczyk, P.B., Jones, S.W. and Mistretta, C.M. Age-related differences in salt taste acuity. J. Gerontology, 34, 834-840, 1979.

11. Gutman, D. and Ben-Aryeh, H. The influence of age on salivary content and rate of flow. Int. J. Oral Surgery, 3, 314-317, 1974.

12. Hambidge, K.M. Increase in hair copper concentration with increasing distance from the scalp. J. Am. Clin. Nutr., 26, 1212-1215, 1973.

13. Henkin, R.I., Graziadei, P.O.G. and Gradley, D.F. The molecular basis of taste and its disorders. Ann. Intern. Med., 71, 791-821, 1969.

14. Hermel, J. Schonwetter, S. and Samueloff, S. Taste sensation and age in man. J. Oral. Med., 25, 39-42, 1970.

15. Hinchcliff, R. Clinical quantitative gustometry. Acta Otolaryngol., 49, 453-466, 1958.

16. Holden, J.M., Wolf, W.R. and Mertz, W. Zinc and copper in self-selected diets. J. Am. Dietet. Assoc., 75, 23-28, 1979.

17. Kimbrell, G. and Furchtgolt, E. The effect of aging on laboratory threshold. J. Geront., 18, 364-365, 1963.

18. Klevay, L.M. Hair as a biopsy material. I. Assessment of zinc nutriture. J. Am. Clin. Nutr., 23, 284-289, 1970b.

19. Klevay, L.M. Hair as a biopsy material. II. Assessment of copper nutriture. J. Am. Clin. Nutr., 23, 1194-1202, 1970a.

20. Langan, M.J. and Yearick, E.S. The effects of improved oral hygiene on taste perception and nutrition of the elderly. J. Gerontol., 31, 413-418, 1976.

21. Mäkila, E. Oral health among the inmates of old people's homes. Salivary secretion. Proc. Finn. Dent. Soc., 73, 64-69, 1977.

22. Marshall, M.W., Iacono, J.M., Young, C.W., Washington, V.A., Slover, H.T. and Leaply, P.M. Composition of diets containing 25 and 35 percent calories from fat. J. Am. Dietet. Assoc., 66, 470-481, 1975.

23. Meyer, J., Golden, J.S., Steiner, N. and Necheles, H. The ptyalin content of human saliva in old age. Am. J. of Physiology, 119, 600-602, 1937.

24. Pangborn, R.M., Witherly, S.A. and Jones, F. Parotid and Whole Mouth secretion in Response to Viewing, Handling and Sniffing Food. Perception, 8, 339-346, 1979.

25. Petering, H.G., Yaeger, D.W. and Witherup, S.O. Trace metal content of hair. I. Zinc and copper content of human hair in relation to age and sex. Arch. Environ. Health, 23, 202-207, 1971.

26. Richter, C.P. and Campbell, K.H. Sucrose taste thresholds of rats and humans. Am. J. Physiol., 128, 291-297, 1940.

27. Robson, J.R.K. and Spell, L. Erythrocyte zinc. J. Am. Clin. Nutr., 34, 1983, 1981.

28. Schiffman, S. Food recognition by the elderly. J. Gerontol., 32, 586-592, 1977.

29. Schiffman, S. Changes in taste and smell with age: psychophysical aspects. In: "Sensory Systems and Communications in the Elderly", Aging, Vol. 10. EDS., Ordy, J.M. and Brizzee, K., pp. 227-246, K. Raven Press, New York, 1979.

30. Schiffman, S.S., Hornack, K. and Reilly, D. Increased taste thresholds of amino acids with age. J. Am. Clin. Nutr., 32, 1622-1628, 1979.

31. Schiffman, S. Magnitude estimation of amino acids for young and elderly subjects. In: "Olfaction and Taste VII", Edited by H. Van der Starre. IRL Press, London, pp. 379-383, 1980.

32. Taper, J.L., Burke, D.M., DeMicco, F.J. and Ritchey, S.J. Zinc and copper interaction and retention in elderly human subjects. Fed. Proc., 39, 430, 1980.

33. Trant, A.S., Pangborn, R.M. and Little, A.C. Potential fallacy of correlating hedonic responses with physical and chemical measurements. J. Food Sci., 46, 583-588, 1981.

34. Venstrom, D. and Amoore, J.E. Olfactory threshold in relation to age, sex and smoking. J. Food Sci., 33, 264-265, 1968.

35. Vir, S.C. and Love, A.H.G. Zinc and copper status of the elderly. J. Am. Clin. Nutr., 32, 1472-1476, 1979.

36. Vir, S.C. and Love, A.H.G. Zinc and copper nutriture of women taking oral contraceptive agents. J. Am. Clin. Nutr., 34, 1479-1483, 1981.

37. Wainwright, W.W. Human saliva XV. Inorganic phosphorus content of resting saliva of 650 healthy individuals. J. Dental Res., 22, 403, 414, 1943.

38. White, H.S. Inorganic elements in weighed diets of girls and young women. J. Am. Dietet. Assoc., 55, 38-43, 1969.

39. White, H.S. Zinc content and the zinc-to-calorie ratio of weighed diets. J. Am. Dietet. Assoc., 68, 243-245, 1976.

VII. SUGGESTIONS FOR FURTHER RESEARCH

Sensory research involving the influence of aging and the perceptions of gustation and olfaction has received only limited attention by psychophysicists. Taste thresholds--detection, recognition and olfactory--are well characterized for a variety of food substances in a population of younger individuals, generally 50 years or less. What is needed, therefore, is a concerted effort to tabulate all threshold data in the elderly using modern sensory evaluation procedures and principles. Preferably, these studies should include a younger group of subjects tasting or sniffing the identical substances under similar conditions. Already there are indications of renewed interest in elderly taste threshold work as evidenced by the recent work by Schiffman (1979) on the "Increased Taste Thresholds of Amino Acids with Age".

Suprathreshold studies are equally important in the assessment of sensory decline during the aging process. As stated in the literature review, only one study compared the sensory scaling of suprathreshold concentrations of tastants between the elderly and a younger population directly (Schiffman, 1980). Additional studies are needed to confirm or to elaborate on the present finding of this dissertation that the elderly possess flattened psychophysical functions for sucrose (sweetness perception) and salt. The other two basic tastes-sourness and bitter perception-have yet to be tested in an elderly population.

Additional research on olfaction is necessary to confirm the finding of a "universal" aroma perception in the elderly. Studies based on intensity scaling of a wide variety of food substances should clarify this puzzling phenomenon.

A more reliable index of Zn/Cu status than hair analyses is probably necessary before one can correlate dietary intakes and the mineral nutritional state in individuals. Saliva, enzyme assays or plasma levels of Zn/Cu are candidates for short term indicators of mineral stores. Correlations between these values and sensory studies of gustation or olfaction may prove doubly rewarding.

VIII. APPENDIX

October 15, 1979

TO ALL RESIDENTS OF BURCHAM HILLS:

Thank you for your attention during my ten minute presentation of the taste project. I would also like to take this opportunity to thank Mr. George Redheffer for his enthusiastic support of this study.

We would like to invite all residents to participate in the study conducted by Dr. R. A. Schemmel and Steven A. Witherly. If you would like to join us in what plans to be an exciting project, we ask that you would read the following materials:

1. INFORMATION BULLETIN: everything about the study is contained right there.

2. CONSENT FORM: if you wish to participate, please read the consent form and sign your name and put the apt. number down so that we might be able to contact you.

3. FINALLY: we will need a little information regarding your state of health since this may influence results. This information will be collected privately in your room.

We appreciate your concern in this project. I will be by later to pick up the consent forms and contact each of you individually.

Yours truly,

Steven A. Witherly

Figure I. Letter to all residents of Burcham Hills encouraging participation.

INFORMATION BULLETIN

To all interested subjects . . .:

We would like to invite all persons residing in:_____

to participate in a unique scientific experiment involving the senses of taste and smell. People willing to join our study will be involved in the following:

1. All subjects will taste tomato juice with various levels of salt and taste lemon drink with varying levels of sugar. We ask you to rate how sweet or salty the samples are and how much you like each sample. Samples are not swallowed--only tasted!

2. Subjects will be asked to identify and then rate the intensity of each food odor. Food samples will be in containers that mask visual cues, but allow one to sniff the ten samples.

3. A small bit of hair is needed from each subject; a snip from the back of the neck will prove adequate. Only two samples are needed: one in the beginning and one at the end of the experiment.

4. Finally, we wish to take salivary samples, collected very simply through the use of three cotton dental rolls (Johnson and Johnson No. 2, 1/2 by 3/8 in.) which are placed in the mouth. Subjects merely place them in the mouth, then sniff lemon wedges and taste lemonade. Saliva soaks into the rolls and by immediately re-weighing them, the amount of saliva produced is determined. At the end of the taste-testing we wish to collect a small amount of saliva by having our panelists drool into a plastic container.

5. When the above data have been compiled, the groups of subjects will be broken up into two groups, with one group receiving a mineral supplement and the other receiving a placebo. Neither the subjects nor the investigators will know who receives what. This prevents bias in the final results. The supplementation period will last 2 months, with a pill taken once a day.

6. Finally, all subjects will be retested as outlined above (1-5).

The project will be quite interesting to those of you who have not participated in taste-testing. The study has been designed with your safety in mind, and we do not anticipate any problems.

Information Bulletin - cont.

THANK YOU FOR LISTENING TO US AND READING THIS BULLETIN. IF ANY QUESTIONS ARISE, FEEL FREE TO CONTACT US ANYTIME AT YOUR LEISURE.

Figure II. Information bulletin given to all residents of Burcham Hills.

October 26, 1979

TO OUR FRIENDS IN BURCHAM HILLS:

A few weeks ago a letter was placed in your mailbox in regards to a taste project to be conducted here. Unfortunately, we did not receive as many responses as we had anticipated.

In order to generate additional interest in our study and dispel any fears you may have about it, we are planning to conduct a taste demonstration to be held in the SNACK BAR on Oct. 30 at 9:30 a.m. By doing so, you will have the opportunity to see and taste for yourself the procedures to be followed in our project.

Please come! Everyone is welcome! Previous experience has shown that taste-testing can be a lot of fun, since most people that we have tested said that they hated to see it end. Even if you feel you are unable to taste or have a low level of taste ability, we are interested in having you as a part of our project.

Thank you very much for your time. If interested, please check the "YES" box below and return this portion to Jane Greene by Oct. 29. If not interested, you are more than welcome to stop by and SEE a taste session in progress. Perhaps you will change your mind.

Sincerely,

Steven A. Witherly, Project Director
Michael D. Dauria, Project Coordinator

PLEASE DETACH HERE AND RETURN THIS PORTION TO JANE GREENE

YOUR NAME:_____

YES, I would be interested in participating in your project.

NOTE: If you are one of the few who did respond earlier, please ignore this letter. We will contact you directly.

Figure III. Follow-up information bulletin to residents of Burcham Hills

MEDICAL RECORD: ELDERLY

1. Are you taking any drugs?

2. Are you taking any vitamins?

3. Are you taking any other pills?

4. Do you smoke?

5. Do you have any of these illnesses?

   Diabetes?

   Kidney Disease?

   High/Low Blood Pressure?

6. Is there anything else we should know concerning your medical history?

Figure IV. Medical record form used to assess drug intakes in the elderly prior to Experiment I.

Consent form for participation in study
Michigan State University
Dept. of Food Science & Human Nutrition

1. I have freely consented to take part in a scientific study being conducted by:_____

   under the supervision of:_____

   Academic Title:_____

2. The study has been explained to me and I understand the explanation that has been given and what my participation will involve.

3. I understand that I am free to discontinue my participation in the study at any time without penalty.

4. I understand that the results of the study will be treated in strict confidence and that I will remain anonymous. Within these restrictions, results of the study will be made available to me at my request.

5. I understand that my participation in the study does not guarantee any beneficial results to me.

_____
Signed

_____
Date

Figure V. Consent form signed by all individuals prior to the participation in Experiment I.

DEGREE OF LIKING FOR TOMATO JUICE

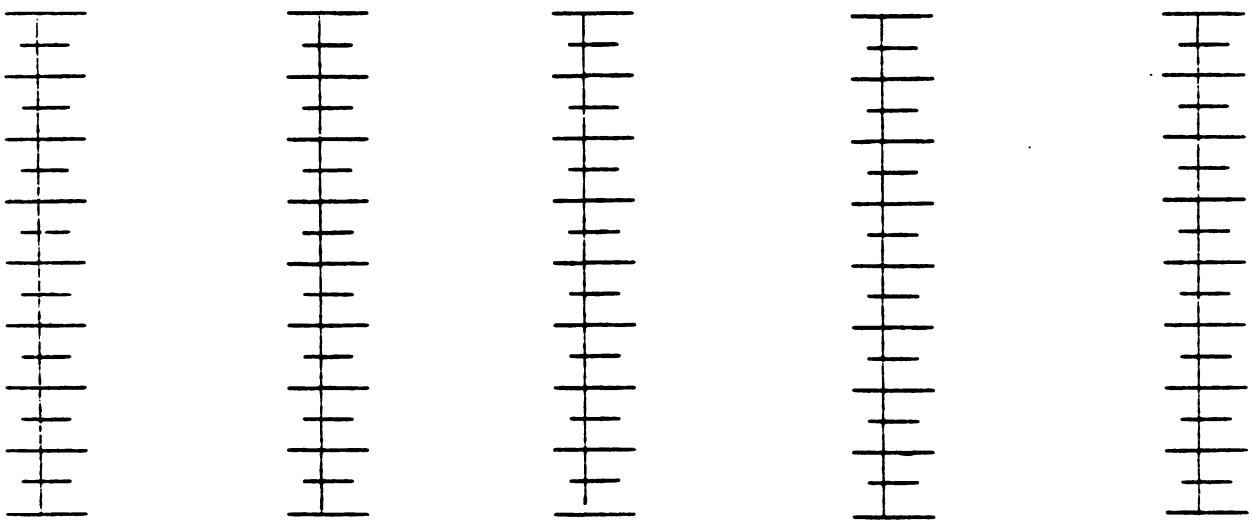
JUDGE:_____ SESSION:_____ DATE:_____

INSTRUCTIONS:

Taste samples in order presented. Place a relatively large volume of tomato juice in the mouth, move it around with the tongue, swallow a small amount and expectorate the remainder. Indicate how much you like each sample by circling one of the horizontal marks on the vertical line. Between each sample, rinse the mouth with distilled water.

like extremely

like extremely



dislike extremely

dislike extremely

Figure VI. Scorecard used in the hedonic evaluation of saltiness in tomato juice.

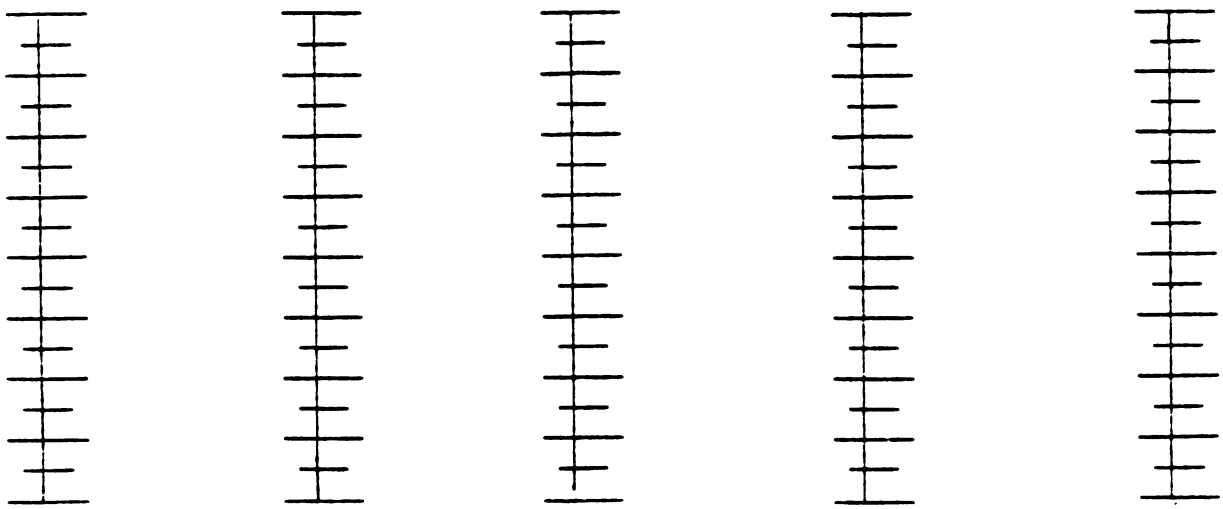
INTENSITY OF SALTINESS IN TOMATO JUICE

JUDGE:_____ SESSION:_____ DATE:_____

INSTRUCTIONS:

Taste samples in order presented. Place a relatively large volume of tomato juice in the mouth, move it around with the tongue, swallow a small amount and expectorate the remainder. Indicate how much you like each sample by circling one of the horizontal marks on the vertical line. Between each sample, rinse the mouth with distilled water.

extremely salty extremely salty



no saltiness no saltiness

Figure VII. Scorecard used in the intensity evaluation of tomato juice saltiness.

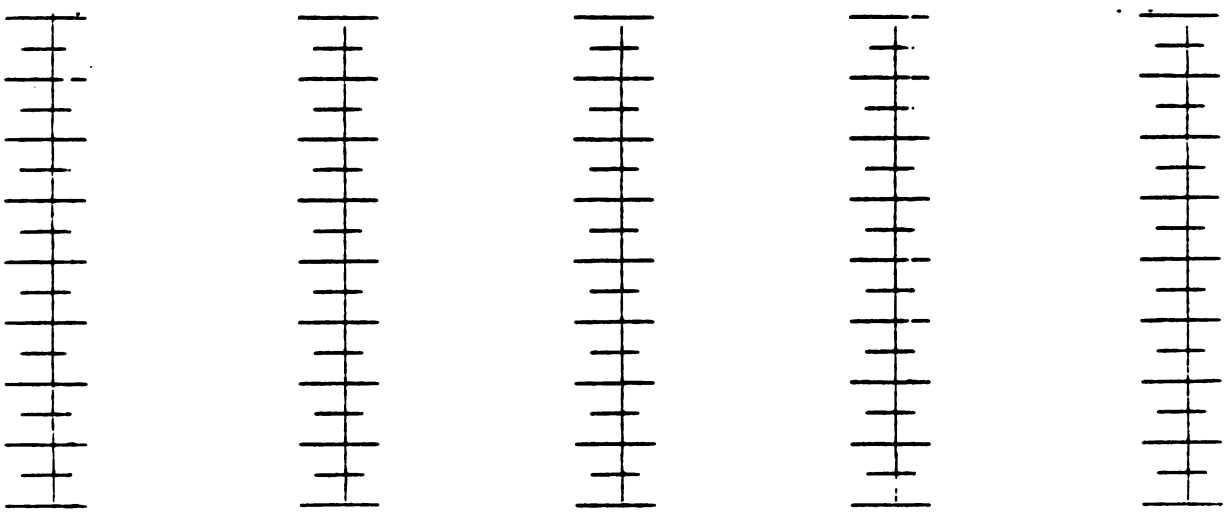
DEGREE OF LIKING FOR LEMONADE DRINK

JUDGE:_____ SESSION:_____ DATE:_____

INSTRUCTIONS:

Taste samples in order presented. Place a relatively large volume of lemonade in the mouth, move it around with the tongue, swallow a small amount and expectorate the remainder. Indicate how much you like each sample by circling one of the horizontal marks on the vertical line. Between each sample, rinse the mouth with distilled water.

like extremely



like extremely

dislike extremely

dislike extremely

Figure VIII. Scorecard used in the hedonic evalutation of sweetness in lemonade.

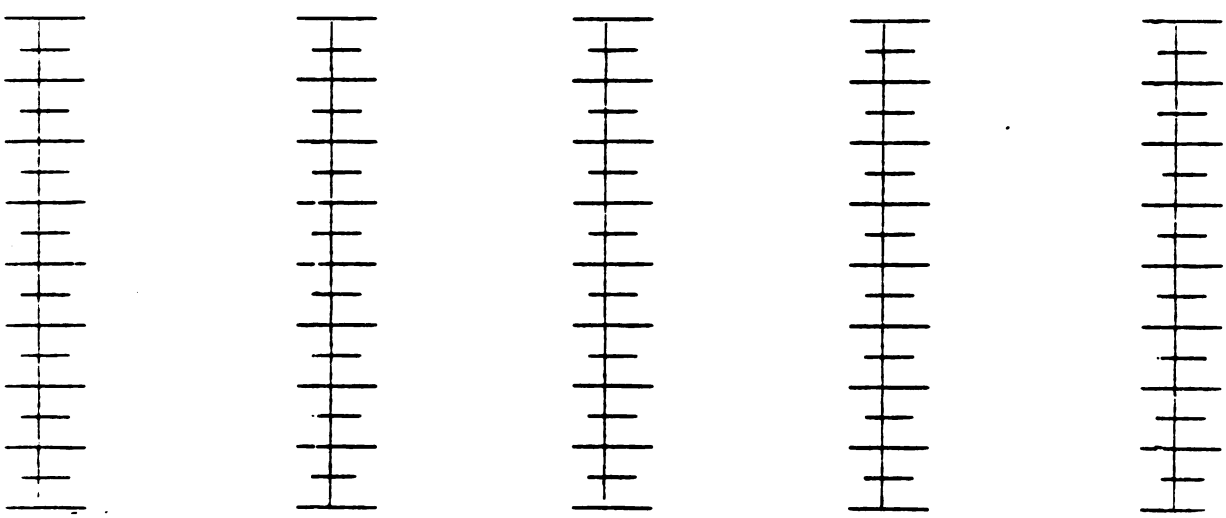
INTENSITY OF SWEETNESS IN LEMONADE DRINK

JUDGE:_____ SESSION:_____ DATE:_____

INSTRUCTIONS:

Taste samples in order presented. Place a relatively large volume of lemonade drink in the mouth, move it around with the tongue, swallow a small amount and expectorate the remainder. Indicate how much you like each sample by circling one of the horizontal marks on the vertical line. Between each sample, rinse the mouth with distilled water.

extremely sweet



no sweetness

extremely sweet

no sweetness

Figure IX. Scorecard used in the intensity evaluation of lemonade sweetness.

Table X. Composition of tomato juice used in Experiment I.

Tomato Juice:

Featherweight Brand[1]

NUTRITION INFORMATION

Calories	35	Fat	0
Protein	2 g	Sodium:	not more than 10 mgs. in 100 g.
Carbohydrates	7 g		

Percent of U.S. recommended daily allowance (U.S. RDA)

Protein	2	Niacin	8
Vitamin A	20	Calcium	2
Vitamin C	2	Iron	4
Thiamin	4	Phosphorus	2
Riboflavin	2	Magnesium	4

[1]Packed for Chicago Dietetic Supplies, Inc., La Grange, IL, 60525, U.S.A.

Table XI. Product information on food products used in the odor recognition, Experiment I, part 3.

Food Product	Description
1. Grape Kool-Aid	Unsweetened soft drink mix. Contains malic acid, mono-calcium phosphate, natural and artificial flavors, vitamin C, artificial color. 6.2 g. General Foods, White Plains, NY, 10625.
2. Nestea, instant tea	100% tea. Nestle Co., Inc. White Plains, NY, 10605.
3. Root Beer Concentrate	Caramel color, water, corn syrup, wild cherry bark extractives and other natural extractives, methyl salicylate and other esters, vanillin and other aldehydes, eugerlol and other esters, gum acacia and gum tragacanth. McCormick, Baltimore, MD, 21202.
4. Kroger Black Pepper	Pure ground black pepper. Pure ground cinnamon. Kroger Co. Cincinnati, Ohio, 45201.
5. Soy Sauce	Water, protein extracts from soy beans, salt, corn syrup and caramel color. La Choy Food Products, Archbold, Ohio, 43502.
6. Cocoa	Sugar, nonfat dry milk, cocoa, corn syrup solids, partially hydrogenated vegetable oil, carboxymethyl cellulose, salt, sodium caseinate, artificial flavor. Carnation Co. Los Angeles, CA, 90036.
7. Ground Coffee	Fine grind. General Foods, White Plains, NY, 10605.

ODOR IDENTIFICATION AND INTENSITY

Name:_____

INSTRUCTIONS: 1. Try to identify the odor in the beaker by sniffing.

2. Then, rate the intensity of the odor by putting a line somewhere between no odor and high odor.

1. SAMPLE:_____, IDENTITY:_____.

NO ODOR — MEDIUM — HIGH

2. SAMPLE:_____, IDENTITY:_____.

NO ODOR — MEDIUM — HIGH

3. SAMPLE:_____, IDENTITY:_____.

NO ODOR — MEDIUM — HIGH

4. SAMPLE:_____, IDENTITY:_____.

NO ODOR — MEDIUM — HIGH

5. SAMPLE:_____, IDENTITY:_____.

NO ODOR — MEDIUM — HIGH

Table XII. Scoresheet used in odor recognition and intensity evaluation of Experiment I, Part 3.

GENERAL GUIDELINES ON HOW TO RECORD FOOD EATEN IN ONE DAY

1. Milk/fruit juice, and other liquids: Record in terms of measuring cups or ounces. (1 measuring cup = 8 ounces.)

2. Butter: Record as level teaspoon. (1 pat of butter = 1 level teaspoon.

3. Sugar: Record as level teaspoons.

4. Eggs: Note whether egg or yolk or white only.

Cereals and Vegetables

Record in terms of measuring cups or tablespoons (1/2 measuring cup equals 8 tablespoons equals 24 teaspoons).

For vegetables

If whole, give size (small, medium, and large). If creamed, record in terms of measuring cup or tablespoons, as above. Note whether fresh, frozen, or canned.

Meats

Record as ounces or level tablespoons if possible. *1 ounce = 2 level tablespoons. Note how cooked. For example, whether it is fried, baked, or broiled.

Other foods

Record as accurately as possible in terms of ordinary household measures. Note flavor (vanilla, choc. or strawberry, etc.) of a baked product and whether it was iced. Brand names are always helpful.

Table XIII. Dietary recording forms used by the college students for their three-day food records in Experiment I.

Name: _____

Time: _____

Complete record of the day's food . . .

----------BREAKFAST----------

Fruit or juice (circle one) Kind: _____, Amount: _____

Cereal Kind:_____, Amount: _____

Milk/with cereal Amount: _____oz. Sugar: _____tsp.

Bread Kind:_____, Number of slices: _____

Butter or margarine (circle one used) Amount:_____tsp.

Other spread Kind: _____ Amount: _____

Egg: Amount:_____ How cooked? _____

Bacon or other meat Kind:_____ Amount: _____oz.

Other foods (please specify):

Kind: _____ Amounts: _____

_____ _____

_____ _____

_____ _____

BETWEEN BREAKFAST AND NOON MEAL, PLEASE RECORD FOODS EATEN

Food (kind)	Amount	Time	Where	With Whom

COMMENTS: _____

_____

_____

_____

Table XIII, (continued)

Name: _____

Time: _____

Complete record of the day's food . . .

----------NOON MEAL----------

Soups, Kind: _____, Amount: _____

Meat/Main Dish, Kind: _____, Amount: _____

Potato: How cooked: _____, Number and size: _____

Vegetable Kind: _____, Amount: _____

Sandwich filling Kind: _____ Amount: _____

Bread Kind: _____ Amount: _____

Butter/margarine (circle one) Amount on bread: _____ tsp.

Salad dressing Kind: _____, Amount: _____

Milk Kind: _____, Amount: _____

Other beverages Kind: _____, Amount: _____

Dessert Kind: _____, Amount: _____

Other foods, please specify:

Kind: _____ Amounts: _____

_____ _____

_____ _____

BETWEEN NOON AND THE EVENING MEAL, PLEASE SPECIFY WHAT YOU ATE:

Food (Kind)	Amount	Time	Where	With Whom

COMMENTS: _____

_____

_____

_____

Table XIII, (continued)

Complete record of the day's food . . . Name: _____

Time: _____

----------EVENING MEAL----------

Meat or Main dish: Kind: _____, Amount: _____

Potato: How cooked? _____, No. and size: _____

Vegetables: Kind: _____ Amount: _____

_____ _____

_____ _____

Bread: Kind: _____, No. of slices: _____

Butter or margarine on bread?_____ Amount: _____tsp.

Gravy: _____, Amount:_____

Salad dressing: Kind: _____, Amount: _____oz.

Milk or other beverages: Kind:_____, Amount: _____oz.

Dessert: Kind:_____, Amount: _____

OTHER FOODS: Kind:_____ Amount: _____

_____ _____

_____ _____

BETWEEN EVENING MEAL AND BEDTIME, PLEASE RECORD WHAT YOU ATE:

Food (Kind)	Amount	Time	Where	With Whom

Comments: _____

_____

_____

Table XIII, (continued)

Page _____

Coding Form For
Project Unlimited

Coder _____

Date _____

Char	Family ID	Person ID	Day	Meal	Loc.
L					

Char	Item	Measure	Quantity
M			
M			
M			
M			
M			
M			
M			
M			
M			
M			

Table XIV. Actual coding form used to record dietary intakes of subjects in Experiment I.

Table XV. Actual food items served during the three-day dietary recording of the elderly in Experiment I.

Menu for 3 Day Food Intake Analysis*

A. Breakfast

*Juices (orange, cranberry, prune, apple, grape, grapefruit, pineapple)
maple syrup
sugar (regular)
Sweet 'n Low (substitute)
Zesta saltines
Keebler-Waldorf low-salt crackers
Tea (Salada)
coffee (regular)
Sanka decaf. coffee
CoffeeMate
*Milk (whole, skim, Farm Maid cultured buttermilk)
hot chocolate (Carnation)
butter (regular and low-salt)
Fleischman's corn oil margarine
fruits (banana, apple, orange, prunes--see lunch/dinner also)
Bread (Holsum white and wheat)
ketchup/mustard/honey (Nifda brand)
cold cereals (Rice Krispies, Cornflakes, Shredded Wheat, Raisin Bran, Special K, All Bran)
hot cereals (Nabisco Instant Cream of Wheat, Quaker Instant Oatmeal w/raisins, Malto-Meal)
french toast
Sausage links
eggs (scrambled, poached, sunny side-up, etc. Chef's Pride Frozen brand)
danish roll
jams (Nifda assorted brands)

B. Lunch

fruit cocktail
soups (Pea, Beef Barley, Cream of Mushroom)
**Sloppy Joe (recipe below)

*Foods of those who ate out or in their rooms are not included on these lists.

**Sloppy Joes

10 lb. Ground Beef (beef and onion)	2 t. dry mustard
8 oz. onion, chopped	2 t. worc. sauce
1 qt. tomato puree	2 t. paprika
2 t. salt	2 t. chili powder

Table XV, (continued)

cottage Cheese Fruit Plate
buttered carrots
chocolate pudding
Spanish rice w/ground beef
tuna salad sandwich
tossed salad with bacon bits
cherry crisp
salad Dressings (French, Gourment--Chadalee Farms, Oil and Vinegar, Creamy Italian, Thousand Island)
hot dog and bun w/slice of pickle
baked beef hash
baked beans
spiced peach salad (jello with peaches)
cherry cake
apple sauce
peaches

C. Dinner Menu

molded vegetable salad (with apricots)
swiss steak with gravy
liver and onions
french fries
ice cream (vanilla and chocolate)
toppings for ice cream (strawberry and chocolate)
wax beans
peas (buttered)
coleslaw
chicken fried steak
lamb pattie with mint jelly
new boiled potatoes
chilled apricots
gingerbread with whipped topping
cottage cheese
beef stroganoff with noodles
filet of fish with tartar sauce
carrot fingers
pears
green beans

Table XVI. Basic measurements and ladle equivalents used by the Burcham Hills staff to portion-out food items to the elderly during the 3-day dietary food recall.

Basic Measurements

Scoop #:	Weight:
24 (red)	1-1/2 - 1-3/4 oz.
20 (yellow)	1-3/4 - 2 oz.
16 (blue)	2 - 2-1/4 oz.
12 (green)	2-1/2 - 3 oz.
10 (ivory)	3 - 4 oz.
8 (grey)	4 - 5 oz.
6 (white)	6 oz.

Ladle Equivalents

Measure:	Weight:
1/8 c	1 oz.
1/4 c	2 oz.
1/2 c	4 oz.
1 c	8 oz.

Table XVII. Product information on food products used in the odor recognition, Experiment II[1]

Food Product	Description
1. Tuna fish, light chunk (Sun Harbor Industries)	9 1/4 oz. can. Tuna packed in soybean oil, seasoned with vegetable broth and salt. San Diego, Calif. 92113.
2. Tomato paste (Contadina)	12 oz. can. A subsidiary of the Carnation Co., Los Angeles, Calif. 90036.
3. Maple syrup (Camp)	8 fl. oz. bottle. Grade "A", dark amber, 100% natural pure maple syrup. Flessisville, Quebec, Canada.
4. Apple juice (Mott's)	40 Fl. oz. bottle. Natural style apple juice, no sugar or preservatives. Duffy-Mott, Co. New York, NY 10017.
5. Garlic powder (Kroger)	2-7/8 oz. canister. Pure garlic powder. Cincinnati, Ohio 45201.

[1]The three other food items--pepper, lemon, and grape--are described in Study I, and Table XI, Appendix.

TO THOSE WHO PARTICIPATED IN THE TASTE SESSION:

We would like to take this opportunity to thank you for your time on Tuesday morning. We hope you will join us for additional sessions to begin next Monday, November 5. These sessions will then be held every Monday, Wednesday, and Friday between 2:00 - 4:00 p.m. in the Snack Bar. Please, we need your help!

We need to test you three times next week, once on each of the three days as indicated above. If you can't make it each time, please don't worry. We can always make other arrangements to suit your schedule.

We will be calling you soon to remind you about the taste sessions and answer any questions you may have about them or the study itself.

Thank you again for your cooperation. This taste experiment means a great deal to us, and we promise you a "good time".

Sincerely,

Steve and Mike

NOTE: If any of your friends would like to join us next week and were reluctant the first time, please invite them along. It's not too late to get involved!

Figure XVIII. Handout distributed to the elderly participants near the end of Experiment II.

From: Steven A. Witherly and Michael D. Dauria

Dear Taste Panelist:

As you may know, the end of the taste panel draws near, and Mike and I would like to thank you for your participation thus far. All that is left is the salivation testing, which is the most interesting part of the study. It involves the collection of a little hair and saliva. For the saliva study, we will call each of you individually to come to the Snack Bar because we can only handle a few people at a time. The times will be about the same:

M-W-F BETWEEN 1:00 and 4:30 STARTING WEDNESDAY, NOVEMBER 28

At the end of the taste study, we hope that you will join us and try the zinc/copper supplement that may improve your senses of taste and smell. All you do is take a tablet of zinc and copper once a day for one month. These tablets are safe, non-toxic, and have no known side effects. Mike and I have taken them for some time now with NO problems whatsoever.

We hope that you will continue with the study and try the zinc and copper. Remember you can stop at any time, but we would greatly appreciate your continued support of our project. If you decide to do so, Mike and I will check back with you weekly and see how your taste and smell senses are doing.

I appreciate your support of my Ph.D. Dissertation Project. Without you, research and progress involving Senior Citizens comes to an abrupt halt.

Thank you again,

Steve and Mike

Figure XIX. Handout given to all elderly participants of Experiment II, near the end of the olfactory studies.

Table XX. Anthropometric data on college students participating in Experiment I and II.

Subject	Gender[1]	Age	Height (cm)	Weight (kg)	Triceps skinfold(mm)	% Fat[2]
1	F	25	168	58	25	33.7
2	F	22	163	52	16	26.7
3	F	22	155	45	18	28.5
4	F	19	170	57	22	31.6
5	F	23	168	59	21	30.9
6	F	28	163	56	23	32.3
7	F	30	173	61	19	30.6
8	F	34	178	73	24	33.9
9	F	21	160	52	25	33.7
10	F	24	173	58	72	31.6
11	F	21	157	59	25	33.7
12	F	31	173	59	20	31.4
		25.0±4.7	166.8±7.2	57.4±6.6	12.0±14.8	31.6±2.2
13	M	23	188	82	8	14.7
14	M	25	188	79	9	15.9
15	M	33	180	79	19	23.5
16	M	22	185	79	16	21.8
17	M	23	183	77	14	20.4
18	M	29	180	61	10	16.9
19	M	25	178	76	12	18.8
20	M	28	178	70	11	17.9
21	M	25	168	54	11	17.9
22	M	24	175	69	20	24.1
23	M	22	185	82	8	14.7
24	M	25	173	68	17	22.4
25	M	26	188	73	9	15.9
		25.4±3.1	180.7±6.3	73±8.4	12.6±4.2	18.8±3.3
Mean:DS		25.2±3.9	174±9.8	65.5±10.9	16.6±6.2	24.9±7.1

[1]Female (F); Male (M)
[2]As calculated from equations by Durnim and Womersley (1974), see Appendix, Table XXVI.

Table XXIA. Anthropometric data on elderly subjects participating in Experiment I and II.

Subject	Gender	Age	Height (cm)	Weight (kg)	Triceps skinfold (mm)
1	F	95	159	40	14
2	F	85	158	48	28
3	F	84	159	41	18
4	F	90	154	61	20
5	F	80	158	76	38
6	F	76	156	66	35
7	M[1]	90	162	68	25
8	F	82	153	68	38
9	F	81	158	75	28
10	F	82	150	44	14
11	F	83	144	58	21
12	F	74	170	57	15
13	F	79	155	69	34
14	F	90	157	80	35
15	F	84	159	83	37
16	F	79	158	66	35
17	F	80	162	66	28
18	F	90	148	49	22
19	F	87	156	65	22
20	F	85	173	74	23
21	F	80	157	53	18
22	F	80	160	50	17
23	M[1]	80	178	84	14
24	F	79	158	49	17
25	F	82	155	58	25
Mean: DS		83±5.0	158±7.0	62±12.9	25±8.0

[1]Note: The two males averaged--85.0±7.1 years; 170.0±11.3 height; 76.0±11.3 weight; and 19.5±7.8 triceps skinfold.

Table XXIB. Anthropometric data on the elderly subjects participating in Experiment III.

Subject	Gender	Age	Height (cm)	Weight (kg)		Triceps skinfold (mm)
				Before	After	
3	F	84	159	41	43.2	18
6	F	76	156	66	61.2	35
7	M	90	162	68	70.6	25
8	F	82	153	68	69	38
12	F	74	170	57	57.2	15
15	F	84	159	83	81.4	37
21	F	80	157	53	52.2	18
25	F	82	155	58	57	25
Mean:SD		81.5±5.0	158.8±5.3	62±13/61.5±11.9		26.4±9.2

Table XXII. Comparison of the anthropometric data of college students and elderly subjects in Experiment I.

Group	Gender	Age	Height (cm)	Weight (kg)	Triceps skinfold	% Fat[1]
College (n-25)	12 female 13 male	25.2±3.9 (19-34)	174±9.8	65.5±10.9	16.6±6.2	25.0
Elderly (n-25)	23 female 2 male	83.0±5.0 (74-95)	158±7.0	62.0±12.9	25.0±8.0	

[1]Based on regression equation for prediction of body fat developed by Durnim and Womersley (1974)

Table XXIII. Answers to the following questions posed in the medical record survey of the elderly.

---

1. Are you taking any drugs?

   No: 6/25 (24%); Yes: 76%

2. Are you taking any vitamins?

   No: 17/25 (68%); Yes: 32%

3. Are you taking any other pills?

   No: 25/25 (100%)

4. Do you smoke?

   No: 25/25 (100%)

5. Do you have any of these illnesses?

   Diabetes? (2/25: (8%) (1 borderline, 1 severe)

   Kidney Disease? 1/25: (4%) (Kidney stones)

   High Blood Pressure? 8/25: (32%) (2 cases slight)

6. Other past diseases: arthritis (3/25); skin cancer (1/25)

Table XXIV. List of drugs mentioned in the medical record of the elderly in Experiment I.[1]

Drug	Manufacturer	Use(s)
Aldactazide	Searle	Diuretic
Antivert	Roerig	Antihistamine
Ascripton	Rorer	Analgesic
Dalmane	Roche	Hypnotic
Deltasone	Upjohn	Anti-inflammatory
Dilitoxin	Purepac	Cardiac-Stimulant
Dyazide	Smith, Kline & French	Diuretic, Anti-Hypertensive (AH)
Hydrodiuril	Merck, Sharp & Dohme	Diuretic, (AH)
Hygroton	USV Pharm.	Diuretic, (AH)
Inderal	Auerot	Hypertension
Lanoxin	Burroughs, Wellcome	Cardiac-Stimulant
Mylicon	Stuart	Flatulence
Notorostat	Parke-Davis	Myocardial ischemia
Peritrate	Warner/Chilcott	Angina Pectoris
Persantine	Boehringer Ingelheim	Angina Pectoris
Prednisone	Forest	Anti-inflammatory
Proloid	Warner/Chilcott	Thyroid extract
Quinamm	Merrel-National	Muscle cramps
Stress-Tabs	Lederle	Vitamins & Calcium
Thiuretic	Parke-Davis	Diuretic
Triavil	Merck, Sharp & Dohme	Tranquilizer
Tranicon	Dista	B_{12} intrinsic factor
Vasodilon	Mead Johnson	Cerebral vascular

[1]Information derived from the Physician's Desk Reference, 1979.

Table XXV. Comments by elderly subjects during the course of their Zn/Cu supplementation period in Experiment III.

Subject	First Week (Feb. 7, 1980)	Second Week (Feb. 14, 1980)
1	No change in food taste; pills are like "horse pills".	Maybe more moisture in the mouth now. I think it's improved.
2	Might have a change of taste; can't say for sure.	No change--maybe more moisture.
3	I haven't noticed anything.	Still alive.
4	If we get in the Guiness Book, I want to be in it.	No problems.
5	Haven't noticed anything.	We're taking our pills.
6	Just taking them regularly.	We're taking our pills.
7	No comments.	No problems.
8	I haven't noticed anything.	Maybe I'm imagining it, but I think I'm smelling better than before.

Table XXV, (continued)

Subject	Third Week (Feb. 21, 1980)	Fourth Week (Feb. 28, 1980)
1	Feeling fine, everything tastes the same.	For a few days, the foods I like taste better.
2	Feeling fine, everything tastes the same.	We're just taking the pills.
3	Nothing at all.	No change.
4	Feeling okay; haven't noticed any special differences.	The sense of smell may be better, other than that, just taking the pills.
5	Just taking the pills religiously and don't notice any difference.	No change--just taking the pills.
6	Just taking the pills religiously, with no changes.	No change-just taking the pills.
7	Nothing.	No change.
8	Can't see any improvement.	I can't see if there is any difference.

Table XXV, (continued)

Subject	Fifth Week (Mar. 6, 1980)	Sixth Week (Mar. 13, 1980)
1	Nothing.	No change, but it was worth a try.
2	Nothing.	I don't realize I'm taking anything.
3	Nothing.	No--guess not.
4	No.	I don't think so.
5	No.	Haven't noticed any change at all.
6	I don't know of anything.	I have more saliva; have to wipe my lips all the time.
7	No.	None that I know of.
8	Can't see if it makes any difference.	No change; I can't say there is any difference.

Table XXV, (continued)

Subject	Week Seven (end) March 20, 1980
1	No change.
2	No change.
3	No comment.
4	Not a thing.
5	No change.
6	Still have lots of saliva.
7	No change.
8	No change.

Table XXVI. Equations by Durnim and Womersley (1974) used to predict % body fat from a triceps skinfold measurement.

Males:	92 Ss, 20-29 yr:	D = (1.1131 - 0.0530) log TS
	34 Ss, 30-39 yr:	D = (1.0834 - 0.0361) log TS
Females:	100 Ss, 20-29 yr:	D = (1.1319 - 0.0776) log TS
	58 Ss, 30-39 yr:	D = (1.1176 - 0.0686) log TS
	48 Ss, 40-49 yr:	D = (1.1121 - 0.0691) log TS

% Fat = 100 (4.95/D - 4.50)

Example: #1 Female. Triceps = 25 mm
Age 25

1) D = (1.1319 - (0.0776 Log + S (25))
= 1.02

% Fat = 100 (4.95/1.02 - 4.50)
= 33.67% Fat