THE EFFECTS OF ORANGE JUICE ALONE AND IN COMBINATION WITH VARIOUS NUTRIENT CONCENTRATES ON CERTAIN BLOOD LEVELS OF ADOLESCENTS

A Thesis

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by

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INTRODUCTION

In a previous study conducted in the Nelda Childers Stark Laboratory for Human Nutrition Research, the Texas Woman's University, on pre-school and elementary school children who were fed orange juice, significant increases were found to take place in the levels of their blood plasma ascorbic acid, and carotene (29). A comparable increase did not occur with the levels of vitamin A.

In order to obtain further information about the effect of orange juice on the levels of these vitamins in the blood, the author has participated in a study using teenagers as subjects. In one part of the study, the diets of high school boys and girls were supplemented with orange juice; and in a second part, the diets of freshman university women were supplemented with various combinations of orange juice and certain nutrients, the latter including ascorbic acid, vitamin A, B-complex vitamins, and protein. In both parts of the study, tests on the blood plasma levels of ascorbic acid, carotene, and vitamin A were made among other biochemical analyses, including the cholesterol content of the blood serum. The recent interest which has developed in cholesterol as a causative agent in vascular disease has prompted the addition of the determination of this blood component to the study.

OBJECTIVES

Thus the study was designed to determine:

(1) the blood levels of ascorbic acid, carotene, vitamin A, and cholesterol of some Texas high school students who were not receiving dietary supplements;

(2) the effect, if any, of orange juice on the level of ascorbic acid in blood;

(3) the effect, if any, of orange juice on the level of vitamin A in the blood;

(4) the effect, if any, of orange juice on the level of carotene in blood;

(5) the effect, if any, of orange juice on the level of cholesterol in blood; and

(6) the effect, if any, of various combinations of orange juice and various specific nutrients on ascorbic acid, vitamin A, B-complex vitamins, and protein, on the levels of ascorbic acid, vitamin A, carotene, and cholesterol in the blood of university women students under 20 years of age.

REVIEW OF LITERATURE

The recently published work done by Mack et al (29) with pre-school and elementary school children who were fed orange juice sets forth the effects of orange juice on the

levels of ascorbic acid, vitamin A, and carotene in the blood. These authors have discussed the effects of orange juice in terms of its known nutritive components and their possible contributions to the results of the feeding program, including the changes, if any, on the blood levels under consideration.

The results of experimentation on the effects of nutrients on the blood levels of cholesterol are abundant in recent scientific as well as popular literature, particularly when the research involves the feeding of fats or cholesterol itself, or both. This study, however, was not concerned with the ingestion of fats or cholesterol, but only with any possible changes in blood serum cholesterol which might be effectuated through the feeding of orange juice or of the nutrients fed in various parts of this investigation.

Green et al (19) reported that, when rats were depleted of vitamin A, their plasma and liver cholesterol levels showed no significant changes from that of the control rats. They concluded that, in a quantitative sense, cholesterol metabolism is not responsive to vitamin A depletion.

Hsu and Chow (22) reported that a deficiency of vitamin B_{12} induced hypercholesterolemia. This result is contrary to that of an experiment described by Olivi and Donnasibilla (38) in which 20 gammas of vitamin B_{12} were administered intramuscularly to 21 children. Their results in-

dicated that cholesterolemia generally was decreased, and they suggested that the effect on cholesterol was probably mediated through the effect of the vitamin on intestinal bacteria, some of which are known to attack cholesterol.

Experiments with nicotinic acid were reported by Lothman (26) in which rabbits were fed five milligrams of nicotinic acid per kilogram of body weight. His results showed that the vitamin had no influence on the level of blood cholesterol.

In an experiment in which human beings were fed nicotinic acid in tablet form, Altschull et al (1) reported that the serum cholesterol decreased. They maintained that the decrease would depend upon the initial cholesterol level because the results showed that hypercholesterolemia levels are more likely to be affected than normal levels.

Lothman (26) gave rabbits one milligram of thiamine per kilogram of body weight. He concluded that thiamine given in conjunction with potassium iodide lowered blood cholesterol.

Greenberg and Rinehart (20) reported that feeding Rhesus monkeys a low or moderate fat diet containing one per cent of cholesterol resulted in a greater cholesterolemia in a pyridoxine deficient group than in a control group. This increase occurred despite the fact that the cholesterol in-

take of the controls was two to four times greater than that of the deficient animals.

In an experiment designed by Loewe et al (25), cholesterol-fed rabbits were maintained on low calorie, high protein diets; and, while the incidence of atherosclerosis was not reduced in these animals, slight decreases in serum cholesterol levels were observed.

Mayer and his coworkers (30) increased the protein content of the diets of groups of rats on a normally hypercholesterolemic regimen (cholesterol and cholic acid). They reported that increasing the protein content of the diet decreased serum cholesterol.

Cholesterol seems to be a raw material for the synthesis of adrenal cortex hormones (7). Also it has been noted that there seems to be a hyposecretion of the adrenal cortex in scorbutic guinea pigs (2, 4). This occurrence has led a few investigators into conducting experiments with cholesterol and ascorbic acid.

Booker et al (12, 13), in conjunction with Robinson et al (39) in a series of experiments with dogs, rats, and guinea pigs, reported that upon the administration of ascorbic acid the serum cholesterol level rises. The authors reported that with the increase in serum cholesterol, there was a decrease of adrenal cholesterol and an increase

of adrenal ascorbic acid. They suggested that the hypercholesterolemia might have resulted from an adrenal response to the storage of ascorbic acid in the gland. The authors also felt, however, that this hypothesis was not adequate to account for the acute hypercholesterolemia that followed ascorbic acid administration, since the rise of serum cholesterol probably is greater than the amount of cholesterol released by the adrenals during the period of ascorbic acid storage. In a later paper the authors expressed the belief that the hypercholesterolemia might result from the liver release of cholesterol which may be related to adrenal hormone action (11).

Becker et al (6) reported that severely scorbutic guinea pigs incorporated six times as much C^{14} from acetate-1-C^{14} into adrenal cholesterol as did their controls.

Consuelo Mendoza (31) demonstrated that ascorbic acid raised the cholesterol level of normal rabbit blood.

Walker et al (41) made a comparison of the serum cholesterol levels of women of two different age groups. The effect of supplementation of the diet with various levels of ascorbic acid on the serum cholesterol in the two groups also was studied. A slight rise in serum cholesterol with a rise in ascorbic acid was noticed in the older group, but not in the younger group.

Morgan, Gillum, and Williams (34), however, in a study conducted with aging human subjects, reported that there seems to be no relationship between blood cholesterol and blood ascorbic acid levels.

PLAN OF PROCEDURE

Two different groups of experimental subjects participated in this study. Since they underwent different supplementations, they will be discussed separately. One group of subjects consisted of high school boys and girls which henceforth will be designated as "high school subjects"; the other group, which consisted entirely of freshman university women at Texas Woman's University, will be known as "university subjects." All subjects, regardless of group, were tested initially and again after a period of supplementation.

HIGH SCHOOL SUBJECTS

The subjects used in this section of the experiment were students of schools located in Amarillo, Sanger, Krum, Ponder, Denton, and Dallas, Texas.

The Amarillo group consisted of 19 males and 19 females. The mean age of the females was 13.7 years and of the males, 13.8 years. Hence, the mean age of the entire group was 13.8 years.

The boys and girls from Sanger had a mean age of 13.5 years. The 12 girls had a mean age of 13.6 years, and the 18 boys of 13.4 years.

Six males and one female from Denton were included in the study, who together had a mean age of 14.3 years.

The subjects from Amarillo, Sanger, and Denton composed the experimental group (those receiving orange juice at school). In this group there was a total of 32 females and 43 males. The mean age of the females was 13.7 years, and of the males this also was 13.7 years. Thus the overall mean age for the 75 subjects was 13.7 years.

These subjects were tested late in the fall of 1957. After their initial test, a daily supplement of 10 ounces of orange juice was served to them daily while at school. This dietary supplementation continued through the remainder of the school year, allowing time in the spring for retesting.

Another group of boys and girls from the schools in Ponder, Krum, and Dallas received no orange juice, and served as controls.

From Ponder there were 12 females, with a mean age of 13.5 years, and 10 males with the same mean age, which brings the mean age of the Ponder group to 13.5 years.

Other controls were students in the high school in Krum. There were 12 females with a mean age of 13.8 years, and nine males with a mean age of 13.7 years in this group. The mean age of the Krum group was 13.7 years.

Seven additional control subjects were enrolled in a private school in Dallas. They were males; and they had an average age of 17.3 years.

This brings the control group to a total of 52 subjects, with a mean age of 14.2 years. The entire group was composed of 24 females with a mean age of 13.7 years, and 28 males with a mean age of 14.7 years.

UNIVERSITY TEEN AGE SUBJECTS

The subjects of this group who completed the study were composed of 85 freshman women enrolled chiefly in the College of Household Arts and Sciences at Texas Woman's University in Denton, Texas. They had a mean age of 18 years. The subjects were divided into five different groups; and each group was supplied with dietary supplements as described below. As was the case with the high school group, these university subjects were tested once, given their supplements for an intervening period during the 1957-1958 academic year, for six days a week, and then retested.

Group I

There was a total of 16 girls in this supplementary group; and they received 10 ounces of orange juice daily for the period of the study.

The mean age of this group was 18 years.

Group II

There were 11 university women in this group, with a mean age of 18 years. Their supplementation consisted of Mellorine, a vitamin A capsule, and a capsule containing B-complex vitamins and ascorbic acid.

Group III

The 24 women in this group were given Mellorine, a vitamin A capsule, a vitamin B-complex capsule containing vitamin C, and also 10 ounces of orange juice.

Their mean age was 18 years.

GROUP IV

The 13 college women in this group received only the Mellorine and the vitamin B-complex capsule with vitamin C.

Their mean age was 18 years.

GROUP V

The 21 university women in this group received 10 ounces of orange juice, Mellorine, and a vitamin B-complex capsule with vitamin C.

Their mean age was 18 years.

The subjects were kept on experiment for slightly more than one school semester. During the last month of the study, 10 ounces of orange juice were added to the daily supplementation of Groups II and IV. Hence, all groups received orange juice during this final period.

A station was set up in one of the women's dormitories at which girls received their supplements according to their group membership. This station was in operation six days of the week. Daily attendance records were kept. Some of the girls failed to appear regularly to obtain their daily supplements. Hence it was decided arbitrarily to drop from the experiment those girls who did not attend at least 50 per cent of the time during the study, and also those who did not attend at least 50 per cent of the time during the last two weeks previous to the final blood tests.

DIETARY SUPPLEMENTS

The orange juice supplied to the university women was identical with that furnished the high school group. Before serving, the orange juice was diluted according to the directions on the can, i.e., one part frozen concentrate with three parts water. Mixed in this manner, 10 ounces of orange juice amounted to the addition of the following food values.

TABLE I

COMMON FOOD VALUES IN TEN OUNCES OF ORANGE JUICE (14)

NUTRIENT	AMOUNT
Energy (Calories)	123
Protein	2.3 gm.
Fat	0.5 gm.
Carbohydrate	31.8 gm.
Vitamin A	137 I. U.
Thiamine	142 mcg.
Riboflavin	53 mcg.
Niacin	0.7 mg.
Ascorbic Acid	118 mg.

These are the more commonly recognized nutrients in orange juice; but these do not take into consideration the lesser known constituents, i.e., inositol (present in notable amounts), biotin, folic acid, pyridoxine, citrates, and the so-called flavonoids, or bioflavonoids. Almost needless to mention is the probability of there being constituents in

orange juice which may be of nutritional value, but which as yet are unknown.

The Mellorine was purchased from a local dairy. There were three and one-half fluid ounces supplied daily to the girls assigned this supplement.

TABLE II

AMOUNT OF FAT, CARBOHYDRATE, AND PROTEIN IN THREE AND ONE-HALF FLUID OUNCES OF MELLORINE

	Per Cent	Grams
Fat	10.48	5.94
Carbohydrate . . .	22.04	12.50
Protein	4.01	2.27

A breakdown of the vitamin capsules which were administered is shown below.

TABLE III

COMPOSITION OF VITAMIN CAPSULES

CAPSULES	AMOUNT
Vitamin A[1]	25,000 I. U.
Becotin with C[2]	
Thiamine	10 mg.
Riboflavin	10 mg.
Nicotinamide	50 mg.
B_6	5 mg.
B_{12}	1 mcg.
Ascorbic Acid	150 mg.

[1] Manufactured by Olafsen Vitamins, Inc., Chicago, Illinois

[2] Manufactured by Eli Lilly, Indianapolis, Indiana

METHODS

Blood was obtained from the subjects by venipuncture. The sample was divided into two parts. One part was allowed to clot, after which it was centrifuged, and the serum removed with a dropper and used in the determination of cholesterol. The other part of the blood was oxalated to prevent clotting. It then was centrifuged and

the plasma removed and used in the determination of the ascorbic acid, vitamin A, and carotene.

Cholesterol was determined by a modification of the method of Zak (43). The solutions used were the same as described by this author. The modification made in the procedure is set forth below.

Pipette four ml. of the ferric chloride precipitating reagent into a large test tube and add 0.1 ml. of serum. Rinse the pipette twice with the solution into which the serum has been placed. Then mix the contents of the tube by shaking. After two to five minutes, filter the mixture through a 7.5 cm. Whatman Number 5 filter paper into a smaller test tube. Withdraw 1 ml. of the filtrate and place in the Evelyn colorimeter tube. Add 3.5 ml. of diluting reagent to the contents of the colorimeter tube. Next layer 3 ml. of concentrated sulfuric acid into the colorimeter tube. Mix the tube cautiously. Allow the tube to sit for 30-45 minutes at room temperature and read the absorbance using the 565 millimicron filter.

The standard curve is prepared by pipetting 0, 1, 2, and 3 ml. of freshly prepared working standard into labeled test tubes. Add 4, 3, 2, and 1 ml., respectively, of diluting solution to each of the test tubes. Next add 0.1 ml. of physiologic saline solution to each test tube and

to the balance of the cholesterol working standard. Mix well. Then with 1 ml. pipettes transfer 1 ml. aliquots from the blank and the four standard tubes into Evelyn colorimeter tubes. Add 3.5 ml. of diluting reagent to each colorimeter tube and then layer 3 ml. of the concentrated sulfuric acid into each of them. Mix cautiously and allow to stand for 30 - 45 minutes. Read each standard versus the blank tube using the 565 millimicron filter.

Calculations:

The cholesterol content of the samples was calculated by the following equation:

Cholesterol concentration (mg/100 ml. serum) = $(O.D.)_{sa}$ (K) where $(O.D.)_{sa}$ = optical density obtained for the sample and (K) = a constant.

(K) was calculated by the equation

$$\frac{C_1 + C_2 + C_3 + \ldots C_n}{N}$$

where C was a factor calculated each day from the standard curve data (see below) and N was the number of times the standard curve was run.

$$C = \frac{\Sigma \text{ (concentration) stds.}}{\Sigma \text{ (O.D.) stds.}}$$

where Σ (conc.) stds. = 100 + 200 + 300 + 400 = 1000 and Σ (O.D.) stds. = a summation of the optical densities obtained for the standards of any day.

This method of calculation was used because the standard deviation of differences on 113 samples run in duplicate was reduced by 2.82 mg.

Ascorbic acid was determined by the method of Mindlin and Butler (33).

The vitamin A and carotene were determined by the method of Kimble (23).

PRESENTATION OF DATA

HIGH SCHOOL SUBJECTS

Parts A and B of Table IV show the mean values obtained for serum cholesterol, plasma ascorbic acid, vitamin A, and carotene for all high school subjects distributed as to group (experimental or control), school, and sex. Because of the complexity of the table, the "combined" data were assembled into smaller tables along with the results of the statistical treatment.

Table V summarizes the findings for subjects in the experimental group and in the control group without regard to school. Part A covers male subjects, Part B females, and Part C, both sexes. As can be seen from the table, both sexes in the experimental group decreased in serum cholesterol means by 8.0 mg. In the controls, the males decreased 17.0 mg. and the females 5.0 mg. In the former group, the loss was statistically significant in the females at the 5 per cent level, but not significant in the males. Conversely, in the control group, the males showed a significant decrease at the one per cent level, while the value for the females was not significant.

With regard to plasma ascorbic acid, both sexes contributed to the mean gain shown in the experimental group for this nutrient. The mean blood level of the experimental females increased more than that of the experimental males; their increases in level were 0.49 mg. and 0.26 mg., respectively. Both of these increases were significant at the 1 per cent level of significance. Neither the males nor the female controls showed significant changes in their plasma ascorbic acid levels. Both the males and the females of the experimental group showed significant increases in their plasma vitamin A levels. The level in the males increased from 37.7 mcg. in the first test to 43.8 mcg. in the second test. The females increased their vitamin A level from 33.9 mcg. to 39.9 mcg. between tests. The males of the control group showed a slight decrease in their mean plasma vitamin A level while the level of the females increased slightly. None of the changes in the control groups, however, were significant.

The mean level of carotene in the blood increased between tests in both sexes, both in the experimental and control groups. The level in the male experimental subjects increased from 0.108 mg. to 0.120 mg. between tests, and the level in females increased from 0.101 to 0.114. The control males had a mean level of 0.091 mg. in Test 1 and a mean level of 0.122 mg. in Test 2. The females in this group had

a mean level of 0.086 mg. and increased to 0.118 mg. in the second test. Thus the controls in Test 1 had a lower mean plasma carotene level than the experimental subjects, but reached approximately the same level as the experimental group in the second test. Even so, the increases in both sexes were statistically significant at the 1 per cent level.

Taking all subjects together regardless of sex, the mean cholesterol level of the experimental group decreased from 184 mg. per 100 ml. to 176 mg. per 100 ml. of serum between tests, the change of which was significant at the one per cent level. The controls showed a decrease also, dropping from 195 mg. per 100 ml. on Test 1 to 183 mg. per 100 ml. in Test 2. This decrease was significant at the 5 per cent level.

Plasma ascorbic acid levels increased in both sexes of the experimental group from a mean of 0.93 mg. per 100 ml. in the first test to 1.28 mg. per 100 ml. in the second test. This increase was significant at the one per cent level. The change in the mean value of the plasma ascorbic acid in the controls was small and not significant.

Vitamin A levels of the full experimental group increased from 36.1 mcg. per 100 ml. of plasma in the first test to 42.2 mcg. in the second test. This increase was significant at the 1 per cent level. The controls did not exhibit

a significant change in their mean vitamin A levels between Tests 1 and 2.

Both the experimental and control groups showed positive changes in their mean levels of carotene between tests. The controls showed a mean gain of 0.032 mg., while the experimental group gained a lesser amount, 0.012 mg. The gains were significant at the 1 per cent level in both groups.

TABLE IV

MEAN BLOOD VALUES FOR HIGH SCHOOL SUBJECTS

EXPERIMENTAL

lood ponent	EXPERIMENTAL SUBJECTS											
	AMARILLO			SANGER			DENTON			COMBINED SCHOOLS		
	Male	Fe-male	Both Sexes	Male	Fe-male	Both Sexes	Male	Fe-male	Both Sexes	Male	Fe-male	Both Sexes
LESTEROL ./100 ml.)												
est 1	181	188	185	187	182	185	173	228	180	182	186	184
est 2	169	168	169	175	190	181	191	223	195	174	178	176
ORBIC ID ./100 ml.)												
est 1	1.01	0.86	0.93	0.88	0.92	0.90	1.16	1.50	1.21	0.98	0.86	0.93
est 2	1.21	1.35	1.28	1.29	1.31	1.30	1.15	1.50	1.20	1.24	1.35	1.28
AMIN A g./100 ml.)												
est 1	35.4	37.7	36.5	36.8	27.2	32.9	47.8	41.8	46.9	37.7	33.9	36.1
est 2	48.9	43.5	46.2	37.0	34.2	35.9	48.1	40.8	47.1	43.8	39.9	42.2
OTENE ./100 ml.)												
est 1	0.111	0.102	0.106	0.101	0.097	0.099	0.120	0.151	0.124	0.108	0.101	0.105
est 2	0.127	0.112	0.120	0.109	0.113	0.111	0.131	0.154	0.134	0.120	0.114	0.117

CONTROLS

lood ponent	CONTROL SUBJECTS											
	PONDER			KRUM			DALLAS			COMBINED SCHOOLS		
	Male	Fe-male	Both Sexes	Male	Fe-male	Both Sexes	Male	Fe-male	Both Sexes	Male	Fe-male	Both Sexes
LESTEROL ./100 ml.)												
est 1	191	203	198	187	189	188	198	X	198	192	198	195
est 2	153	179	167	190	203	197	188	X	188	175	193	183
CID g./100 ml.)												
Test 1	0.80	1.09	0.96	0.53	0.90	0.74	1.37	X	1.37	0.89	0.97	0.93
Test 2	0.97	0.92	0.95	0.73	0.82	0.78	1.09	X	1.09	0.94	0.83	0.89
TAMIN A cg./100 ml.)												
Test 1	38.4	34.1	36.1	42.6	43.6	43.2	55.8	X	55.8	44.8	39.5	42.4
Test 2	35.8	39.1	37.6	41.3	44.2	42.9	54.4	X	54.4	43.5	41.6	42.6
ROTENE g./100 ml.)												
Test 1	0.106	0.084	0.094	0.079	0.086	0.083	0.083	X	0.083	0.091	0.086	0.088
Test 2	0.114	0.106	0.109	0.129	0.125	0.126	0.126	X	0.126	0.122	0.118	0.120

ml.)												
	181	188	185	187	182	185	173	228	180	182	186	1
	169	168	169	175	190	181	191	223	195	174	178	1
ml.)												
	1.01	0.86	0.93	0.88	0.92	0.90	1.16	1.50	1.21	0.98	0.86	0.
	1.21	1.35	1.28	1.29	1.31	1.30	1.15	1.50	1.20	1.24	1.35	1.
A ml.)												
	35.4	37.7	36.5	36.8	27.2	32.9	47.8	41.8	46.9	37.7	33.9	36
	48.9	43.5	46.2	37.0	34.2	35.9	48.1	40.8	47.1	43.8	39.9	42
ml.)												
	0.111	0.102	0.106	0.101	0.097	0.099	0.120	0.151	0.124	0.108	0.101	0.1
	0.127	0.112	0.120	0.109	0.113	0.111	0.131	0.154	0.134	0.120	0.114	0.1

OLS

t	CONTROL SUBJECTS											
	PONDER			KRUM			DALLAS			COMBINED SCHOOL		
	Male	Female	Both Sexes	Male	Female	Both Sexes	Male	Female	Both Sexes	Male	Female	Both Sexes
ROL l.)												
	191	203	198	187	189	188	198	X	198	192	198	19
	153	179	167	190	203	197	188	X	188	175	193	18
l.)												
	0.80	1.09	0.96	0.53	0.90	0.74	1.37	X	1.37	0.89	0.97	0.9
	0.97	0.92	0.95	0.73	0.82	0.78	1.09	X	1.09	0.94	0.83	0.8
A ml.)												
1	38.4	34.1	36.1	42.6	43.6	43.2	55.8	X	55.8	44.8	39.5	42.
2	35.8	39.1	37.6	41.3	44.2	42.9	54.4	X	54.4	43.5	41.6	42.6
E ml.)												
1	0.106	0.084	0.094	0.079	0.086	0.083	0.083	X	0.083	0.091	0.086	0.08
2	0.114	0.106	0.109	0.129	0.125	0.126	0.126	X	0.126	0.122	0.118	0.12

TABLE V

SUMMARY OF MEAN BLOOD VALUES, THEIR DIFFERENCES AND STATISTICAL SIGNIFICANCE FOR THE HIGH SCHOOL SUBJECTS

A. MALE SUBJECTS

Test Periods and Statistical Factors	EXPERIMENTAL				CONTROLS			
	Cholesterol (mg.)*	Ascorbic Acid (mg.)*	Vitamin A (mcg.)*	Carotene (mg.)*	Cholesterol (mg.)*	Ascorbic Acid (mg.)*	Vitamin A (mcg.)*	Carotene (mg.)*
Test 1	182	0.98	37.7	0.108	192	0.89	44.8	0.091
Test 2	174	1.24	43.8	0.120	175	0.94	43.5	0.122
Differences	-8	+0.26	+6.1	+0.012	-17	+0.05	-1.3	+0.031
t-value	1.65	2.58	3.44	3.35	3.20	0.48	0.64	3.56
Significance	N.S.**	$P \leq .05$	$P \leq .01$	$P \leq .01$	$P \leq .01$	N.S.	N.S.	$P \leq .01$

B. FEMALE SUBJECTS

Test 1	186	0.86	33.9	0.101	198	0.97	39.5	0.086
Test 2	178	1.35	39.9	0.114	193	0.83	41.6	0.118
Differences	-8	+0.49	+6.0	+0.013	-5.0	-0.14	+2.1	+0.032
t-value	2.10	5.33	5.06	3.43	0.57	1.63	0.28	5.03
Significance	$P \leq .05$	$P \leq .01$	$P \leq .01$	$P \leq .01$	N.S.	N.S.	N.S.	$P \leq .01$

C. BOTH SEXES

Test 1	184	0.93	36.1	0.105	195	0.93	42.4	0.088
Test 2	176	1.28	42.2	0.117	183	0.89	42.6	0.120
Differences	-8	+0.35	+6.1	+0.012	-12	-0.04	+0.2	+0.032
t-value	5.17	5.08	5.37	4.53	2.39	0.53	0.19	5.75
Significance	$P \leq .01$	$P \leq .01$	$P \leq .01$	$P \leq .01$	$P \leq .05$	N.S.	N.S.	$P \leq .01$

* per 100 ml.

** In this table and in all succeeding tables, the entry "N.S." stands for "not significant", meaning that the obtained differences could easily have occurred by chance alone. The entry "$P \leq .05$" means that a difference of the size obtained would occur by chance alone no more than five times in one hundred experiments. Similarly, the entry "$P \leq .01$" means that a difference of the size obtained would occur by chance not more than one time in one hundred experiments.

Table VI shows the percentage distribution of the high school subjects in different class levels depending on the concentration of the respective nutrients in the blood. The classifications were established so that the lower class levels indicate more desirable nutritional status. The classification system used for ascorbic acid, vitamin A, and carotene was designed by Dr. Pauline Beery Mack.

The percentage distribution of the subjects in the designated cholesterol ranges is shown in the table. In the experimental group, 9.3 per cent of the subjects fell in Class 1 in Test 1, while in Test 2, 18.1 per cent fell in this class. A similar trend was shown in the controls between Tests 1 and 2. The greatest percentage of the subjects either in the experimental or in the control group, regardless of test, fell in Class 2, which has a range of 150 to 199 mg. of cholesterol per 100 ml. of serum.

The percentage distribution of the subjects in the designated ranges of ascorbic acid shows that only 37.3 per cent of the subjects were optimum in their ascorbic acid levels in the experimental group at the time of the first test, but that 78.7 per cent were in the optimum classification with respect to this nutrient when the second test was performed. The data show that 22.7 per cent of the subjects in the experimental group fell in Class 5 in Test 1; and, while this class contained only 2.7 per cent of this group

when Test 2 was carried out, there were no great shifts between tests in the percentage distribution of the control subjects with regard to this nutrient.

The percentage distribution of the subjects with regard to vitamin A levels shows that the experimental group had almost 25 per cent fewer subjects in Class 1 than did the control group in the first test. By the time of the second test, however, the per cent of the experimental subjects in Class 1 had increased to a percentage comparable to that of the controls. None of the subjects either in the experimental or control groups was in the lowest vitamin A classification in the second test.

In the experimental group, 70.6 per cent came in carotene Classes 1, 2, and 3, combined, in the second test, while only 50.7 per cent of the group were in these classifications in Test 1. In the same three classes in the first test, there were 38.4 per cent of the controls, while in the second test there were 71.2. Thus there was a gain in the number of subjects in the first three classes both in the experimental and in the control subjects, with a decrease in the number of subjects in the lower classes.

TABLE VI

PERCENTAGE DISTRIBUTION OF THE HIGH SCHOOL STUDENTS IN THE DESIGNATED BLOOD LEVEL RANGES

Classes of Blood Level Ranges	EXPERIMENTAL		CONTROLS	
	Test 1 (per cent)	Test 2 (per cent)	Test 1 (per cent)	Test 2 (per cent)
CHOLESTEROL (mg./100 ml.)				
Class 1 (149 mg. and below)	9.3	18.7	1.9	17.3
Class 2 (150 - 199)	61.3	60.0	69.2	51.9
Class 3 (200 - 249)	26.7	21.3	21.3	25.0
Class 4 (250 - 299)	2.7	0.0	5.7	5.8
Class 5 (300 and over)	0.0	0.0	1.9	0.0
ASCORBIC ACID (mg./100 ml.)				
Class 1 (1.10 mg. and over)	37.3	78.7	36.5	30.8
Class 2 (1.09 - 0.90_	16.0	13.3	7.7	15.4
Class 3 (0.89 - 0.70)	10.7	4.0	21.2	26.9
Class 4 (0.69 - 0.50)	13.3	1.3	19.2	11.5
Class 5 (0.49 and below)	22.7	2.7	15.4	15.4
VITAMIN A (mcg./100 ml.)				
Class 1 (35.5 mcg. and over)	52.0	72.0	76.9	73.1
Class 2 (35.4 - 29.5)	21.3	18.7	15.4	13.5
Class 3 (29.4 - 23.5)	18.7	8.0	5.8	9.6
Class 4 (23.4 - 17.5)	6.7	1.3	0.0	3.8
Class 5 (17.4 and below)	1.3	0.0	1.9	0.0
CAROTENE (mg./100 ml.)				
Class 1 (0.20 mg. and over)	2.7	6.7	1.9	1.9
Class 2 (0.19 - 0.15)	16.0	17.2	0.0	23.1
Class 3 (0.14 - 0.10)	32.0	46.7	36.5	46.2
Class 4 (0.09 - 0.05)	44.0	26.7	51.9	25.0
Class 5 (0.04 and below)	5.3	2.7	9.7	3.8

	(per cent)	(per cent)	(per cent)	(per cent)
CHOLESTEROL (mg./100 ml.)				
Class 1 (149 mg. and below)	9.3	18.7	1.9	17.3
Class 2 (150 - 199)	61.3	60.0	69.2	51.9
Class 3 (200 - 249)	26.7	21.3	21.3	25.0
Class 4 (250 - 299)	2.7	0.0	5.7	5.8
Class 5 (300 and over)	0.0	0.0	1.9	0.0

ASCORBIC ACID (mg./100 ml.)				
Class 1 (1.10 mg. and over)	37.3	78.7	36.5	30.8
Class 2 (1.09 - 0.90_	16.0	13.3	7.7	15.4
Class 3 (0.89 - 0.70)	10.7	4.0	21.2	26.9
Class 4 (0.69 - 0.50)	13.3	1.3	19.2	11.5
Class 5 (0.49 and below)	22.7	2.7	15.4	15.4

VITAMIN A (mcg./100 ml.)				
Class 1 (35.5 mcg. and over)	52.0	72.0	76.9	73.1
Class 2 (35.4 - 29.5)	21.3	18.7	15.4	13.5
Class 3 (29.4 - 23.5)	18.7	8.0	5.8	9.6
Class 4 (23.4 - 17.5)	6.7	1.3	0.0	3.8
Class 5 (17.4 and below)	1.3	0.0	1.9	0.0

CAROTENE (mg./100 ml.)				
Class 1 (0.20 mg. and over)	2.7	6.7	1.9	1.9
Class 2 (0.19 - 0.15)	16.0	17.2	0.0	23.1
Class 3 (0.14 - 0.10)	32.0	46.7	36.5	46.2
Class 4 (0.09 - 0.05)	44.0	26.7	51.9	25.0
Class 5 (0.04 and below)	5.3	2.7	9.7	3.8

UNIVERSITY SUBJECTS

In Table VII, all of the mean values of the blood components which were under investigation are shown under their proper supplementary groups. The results are given for the initial test and also for the second, or final test. The differences between Test 1 and Test 2 are shown, as well as the values which indicate whether or not these differences are statistically significant.

Group I

Initially, the mean serum cholesterol value in this group, which received only orange juice, was 174 mg. per 100 ml. Between Test 1 and Test 2, the value rose 35 mg. to 209 mg. per 100 ml. This rise was significant at the 1 per cent level.

The ascorbic acid level also showed a significant increase for this group. On Test 1, the mean level was 0.82 mg. per 100 ml. plasma, but by the second test the mean ascorbic acid had increased to 1.56 mg. per 100 ml. of plasma.

This university group also showed a rise in plasma vitamin A levels. On the initial test, the mean value was 46.0 mg. per 100 ml. plasma; and at the time of the final test it had risen to 50.5 mg. per 100 ml. This rise was significant at the 5 per cent level.

The group also demonstrated a gain in its plasma carotene levels. From 0.124 mg. per 100 ml. plasma on the first test, the carotene levels increased to 0.158 mg. on the second test. A rise of this magnitude was significant at the 1 per cent level.

Group II

In this group which received Mellorine, vitamin A, and vitamin B-complex with ascorbic acid, the mean cholesterol level showed a slight increase of 12 mg. from Test 1 to Test 2; but this difference was not statistically significant.

The mean ascorbic acid level on the first test rose for Group II from 0.88 mg. per 100 ml. plasma to 1.83 mg. by the second test. This increase was significant at the 1 per cent level.

The vitamin A and carotene values had a tendency to rise from the first test to the second; but the gain was meager and not significant statistically.

Group III

This group, which received all of the supplements used in the study, had results similar to those shown by Group II. In the cases of the serum cholesterol and plasma vitamin A and carotene, there were small increases from Test 1 to Test 2. These changes, however, were not significant.

The ascorbic acid mean value showed an increase significant at the 1 per cent level. The mean blood plasma ascorbic acid was 0.73 mg. per 100 ml. plasma on the first test. A gain of 0.79 mg. was exhibited by Test 2, to give a mean level of 1.52 mg. per 100 ml.

Group IV

In Group IV, which received Mellorine and B-complex with vitamin C, the mean cholesterol level of the students rose from 171 mg. per 100 ml. serum on the first test to 189 mg. per 100 ml. on the second. This gain of 18 mg., however, was not statistically significant.

The mean ascorbic acid level increased from 0.79 mg. per 100 ml. to 1.58 mg. for the first and second tests, respectively. This rise was significant at the 1 per cent level.

The plasma vitamin A and carotene levels did not demonstrate any significant changes from one test to another. Unlike the gains shown in the other groups, however, these nutrients exhibited small losses.

Group V

For Group V subjects who received all of the supplements except vitamin A, the tendency for the cholesterol to increase slightly from the first to the second test was

maintained. Even so, the gain was not of sufficient magnitude to be significant.

The mean ascorbic acid level gained 0.83 mg. from Test 1 to Test 2. The original level was 0.80 mg. per 100 ml. of plasma; and the gain raised the final level to 1.63 mg. per 100 ml. plasma. This increase was significant at the 1 per cent level.

As in the case of Group IV, the vitamin A and carotene levels showed no significant changes. There was a small loss in the vitamin A levels, although the carotene showed no change from one test to another.

As with the high school subjects, a table is presented in which the blood components under investigation were broken down into various levels and the university subjects were placed in their respective ranges and the results reported in percentages. Table VIII gives the results for each of the supplementary groups for both tests in terms of the per cent in the various classes for the different blood levels.

OVERVIEW

Cholesterol

In all of the groups, the largest per cent of the subjects fell in Class 2 for cholesterol, or the 150-199 mg. level, with 56.3 per cent in Group I falling in this level initially. This same group showed a significant increase in the second test; and this brought about a shift to the 200-249 mg. level, or to Class 3. From the first test, when Class 3 contained 25 per cent of the subjects, a jump to 56.2 per cent of the subjects was found on the second test.

Group II showed the largest per cent (45.4) of its subjects at the 150-199 mg. level on the initial test. By the second test, this level had decreased to 36.4 per cent of the subjects and the level of 250 mg. and over had gained nine per cent of the subjects.

Group III had 62.5 per cent of the subjects at the 150-199 mg. level on the first test. The major change between tests fell in the direction of the 200-249 mg. level. This tendency occurred in Group IV, also.

Group V had 42.8 per cent of its subjects at the 150-199 mg. level in Test 1. This increased to 52.4 per cent on Test 2, with the next largest section falling at the 200-249 mg. level (33.3 per cent).

Ascorbic Acid

At the second test all of the groups had increased their mean ascorbic acid values to 1.10 mg. or over. Groups I, II, III, IV, and V had 75.0, 90.9, 79.2,100.0, and 95.2 per cent of the subjects, respectively, at this level. At the time of the second test, there were no subjects found in Classes 4 and 5.

Vitamin A

The majority of the subjects fell in the favorable level of 35.5 mcg. of vitamin A per 100 ml. or over at the initial test. That is, 83.3 per cent to 100 per cent gave values at least this high. For the most part, the second tests showed very little change in this respect. Most of the subjects remained in Class 1.

Carotene

Sixty-two and one-half per cent of the subjects in Group I fell at the 0.10-0.14 mg. level for carotene in Test 1. Twelve and one-half per cent of these subjects had moved to the 0.15-0.19 mg. level by Test 2.

Group II had 45.4 per cent of the subjects in the 0.10-0.14 mg. level in Test 1; and this increased to 63.6 per cent by the second test.

Group III subjects showed the same tendency as Group II, with 45.8 per cent initially in Class 3; increasing to 50.0 per cent on the second test.

On the first test, Group IV had 53.8 per cent of the subjects in Class 2, or within 0.15-0.19 mg. range. The mean carotene level fell on the second test, however, and the largest per cent of the subjects fell to the Class 3 level.

The largest percentage (37.1) of the subjects in Group V were continued in the third carotene classification level on the initial test. By the second test, however, this class had decreased to 23.8 per cent; and Class 2 had gained 28.6 per cent, ending the study with 42.9 per cent of the subjects in this classification.

TABLE VII

MEAN BLOOD VALUES OF BOTH TESTS, DIFFERENCES, AND STATISTICAL SIGNIFICANCE FOR THE UNIVERSITY SUBJECTS

Groups	Test I	Test 2	Differences	t-value	Significance
GROUP I					
CHOLESTEROL (mg./100 ml.)	174	209	+35.0	6.33	$P \leq .01$
ASCORBIC ACID (mg./100 ml.)	0.82	1.56	+0.74	3.81	$P \leq .01$
VITAMIN A (mcg./100 ml.)	46.0	50.5	+4.5	2.68	$P \leq .05$
CAROTENE (mg./100 ml.)	0.124	0.158	+0.034	5.59	$P \leq .01$
GROUP II					
CHOLESTEROL (mg./100 ml.)	182	194	+12.0	1.47	N.S.
ASCORBIC ACID (mg./100 ml.)	0.88	1.83	+0.95	6.79	$P \leq .01$
VITAMIN A (mcg./100 ml.)	46.7	51.3	+4.6	0.981	N.S.
CAROTENE (mg./100 ml.)	0.142	0.150	+0.008	0.717	N.S.
GROUP III					
CHOLESTEROL (mg./100 ml.)	180	199	+19.0	1.91	N.S.
ASCORBIC ACID (mg./100 ml.)	0.73	1.52	+0.79	7.07	$P \leq .01$
VITAMIN A (mcg./100 ml.)	47.5	49.7	+2.2	0.550	N.S.
CAROTENE (mg./100 ml.)	0.144	0.146	+0.002	0.273	N.S.
GROUP IV					
Cholesterol (mg./100 ml.)	171	189	+18.0	1.95	N.S.
ASCORBIC ACID (mg./100 ml.)	0.79	1.58	+0.79	3.10	$P \leq .01$
VITAMIN A (mcg./100 ml.)	48.9	43.4	5.5	1.85	N.S.
CAROTENE (mg./100 ml.)	0.137	0.127	-0.010	1.40	N.S.
GROUP V					
CHOLESTEROL (mg./100 ml.)	193	200	+7.0	0.810	N.S.
ASCORBIC ACID (mg./100 ml.)	0.80	1.63	+0.83	8.10	$P \leq .01$
VITAMIN A (mcg./100 ml.)	45.7	43.4	-2.3	1.01	N.S.
CAROTENE (mg./100 ml.)	0.156	0.156	0.000	0.371	N.S.

TABLE VIII

PERCENTAGE DISTRIBUTION OF THE UNIVERSITY SUBJECTS IN THE DESIGNATED BLOOD LEVEL RANGES

Classes of Blood Level Ranges	GROUP I		GROUP II		GROUP III		GROUP IV		GROUP IV	
	Test 1 (per cent)	Test 2 (per cent)	Test 1 (per cent)	Test 2 (per cent)	Test 1 (per cent)	Test 2 (per cent)	Test 1 (per cent)	Test 2 (per cent)	Test 1 (per cent)	Test 2 (per cent)
CHOLESTEROL (mg./100 ml.)										
Class 1 (149 mg. and below)	18.7	6.3	18.2	18.2	15.6	12.5	23.1	7.7	14.1	4.8
Class 2 (150 - 199)	56.3	37.5	45.4	36.4	62.5	41.7	61.5	53.8	42.8	52.4
Class 3 (200 - 249)	25.0	56.2	36.4	36.4	12.5	33.3	15.4	38.5	28.8	33.3
Class 4 (250 - 299)	0.0	0.0	0.0	9.0	4.7	8.3	0.0	0.0	14.3	9.5
Class 5 (300 and over)	0.0	0.0	0.0	0.0	4.7	4.2	0.0	0.0	0.0	0.0
ASCORBIC ACID (mg./100 ml.)										
Class 1 (1.10 mg. and over)	18.7	75.0	36.4	90.9	20.8	79.2	15.4	100.0	28.6	95.2
Class 2 (1.09 - 0.90)	18.7	18.7	18.2	0.0	16.7	16.7	23.1	0.0	9.5	4.8
Class 3 (0.89 - 0.70)	25.0	6.3	9.1	9.1	12.5	4.1	23.1	0.0	33.3	0.0
Class 4 (0.69 - 0.50)	12.6	0.0	0.0	0.0	8.3	0.0	15.4	0.0	9.5	0.0
Class 5 (0.49 and below)	25.0	0.0	36.3	0.0	41.7	0.0	23.1	0.0	19.1	0.0
VITAMIN A (mcg./100 ml.)										
Class 1 (35.5 [illegible]d	87.6	93.8	100.0	100.0	83.3	95.8	100.0	92.3	90.5	90.5
Class 2 (35.4 - 29.5)	6.2	6.2	0.0	0.0	0.0	4.2	0.0	7.7	4.8	4.8
Class 3 (29.4 - 23.5)	0.0	0.0	0.0	0.0	12.5	0.0	0.0	0.0	0.0	4.8
Class 4 (23.4 - 17.5)	6.2	0.0	0.0	0.0	4.2	0.0	0.0	0.0	4.8	0.0
Class 5 (17.4 and below)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CAROTENE (mg./100 ml.)										
Class 1 (0.20 mg. and over)	12.5	12.5	9.1	9.1	20.8	16.7	0.0	0.0	29.6	19.0
Class 2 (0.19 - 0.15)	6.2	50.0	36.4	27.3	16.7	20.8	53.8	38.4	14.3	42.9
Class 3 (0.14 - 0.10)	62.5	37.5	45.4	63.6	45.8	50.0	30.8	46.2	37.1	23.8
Class 4 (0.09 - 0.05)	18.8	0.0	9.1	0.0	16.7	12.5	15.4	15.4	19.0	14.3
Class 5 (0.04 and below)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

ANALYSIS OF VARIANCE

The Texas Woman's University subjects were divided into five groups and were given different supplementary treatments, each under its own set of conditions. In order to arrive at some indication as to whether or not there were differences between the Test 1 - Test 2 changes exhibited by the groups, analyses of variance were calculated on the blood components under investigation. This particular statistical treatment is useful when there are several groups; otherwise, one might contend with the practical difficulty of pairing two sets at a time, pairing each one with every other one, and testing the reliability of the differences (21).

Table IX shows the values found for the *F* ratio and also gives information as to whether or not the values are significant.

TABLE IX

ANALYSIS OF VARIANCE BETWEEN THE TWO GROUPS FOR THE BLOOD COMPONENTS UNDER INVESTIGATION

A. CHOLESTEROL

Components	Degrees of Freedom	Sums of Squares	Variance
Between Sets	4	7810	1953
Within Sets	80	109301	1357
	$F = \frac{1953}{1357} = 1.44$		N.S.

B. ASCORBIC ACID

Components	Degrees of Freedom	Sums of Squares	Variance
Between Sets	4	5087	1272
Within Sets	80	2760	3450
	$F = \frac{1272}{3450} = 0.369$		N.S.

C. VITAMIN A

Components	Degrees of Freedom	Sums of Squares	Variance
Between Sets	4	1126	281
Within Sets	80	22513	281
	$F = \frac{281}{281} = 1.0$		N.S.

D. CAROTENE

Components	Degrees of Freedom	Sums of Squares	Variance
Between Sets	4	17968	4492
Within Sets	80	86146	1077
	$F = \frac{4492}{1077} = 4.17$		$P \leq .01$

TABLE IX

ANALYSIS OF VARIANCE BETWEEN THE TWO GROUPS FOR THE BLOOD COMPONENTS UNDER INVESTIGATION

A. CHOLESTEROL

Components	Degrees of Freedom	Sums of Squares	Variance
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	$F = \frac{1272}{3450} = 0.369$		N.S.

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	$F = \frac{281}{281} = 1.0$		N.S.

D. CAROTENE

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Between Sets	4	17968	4492
Within Sets	80	86146	1077
	$F = \frac{4492}{1077} = 4.17$		$P \leq .01$

DISCUSSION

HIGH SCHOOL SUBJECTS

Cholesterol

It already has been mentioned that significant decreases were found in the mean serum cholesterol levels between the initial and final tests for the high school groups. The loss, to which both the males and females contributed, occurred not only in the experimental group but also in the control group. In the experimental group, the largest contribution to the decrease came from the Amarillo school, while in the control group the Ponder school supplied the major portion of the loss. Neither of the losses which occurred in the two schools lends itself to a plausible explanation.

The mean cholesterol level of all subjects both of the experimental and control groups, combined, was 188 mg. per 100 ml. of serum on the initial test and 179 mg. per 100 ml. on the final test. The lowest mean value, regardless of sex, school, or test was 153 mg. per 100 ml., which was obtained with the Ponder males in the first test; and the highest was 203 mg. per 100 ml. obtained with the Ponder females in the first test and the Krum females in the second test.

The mean value for all males in the first test was 186 mg. per 100 ml.; and for the females it was 191 mg. per 100 ml. In the second test, the experimental males had a mean value of 174 mg. per 100 ml., and the experimental females a value of 178 mg. per 100 ml. The similar figure for the control males was 175 mg. per 100 ml. and for the control females 193 mg. per 100 ml. About 60 per cent of all subjects appeared in cholesterol Classification 2 (150-199 mg. per 100 ml. serum) either in the first or second test.

Kornerup (23) has reported that 96 children varying in age from one to 16 years had an average serum cholesterol of 209 mg. per 100 ml. Offenkrantz and Karshaw (37), in a series of determinations of the cholesterol content of the blood serum of 250 children, ranging in age from two months to 12 years, found the average to be 183 mg. per 100 ml. Neither Kornerup, nor Offenkrantz and Karshaw noted significant differences between sexes. Bring et al (15), in a study with 15 to 16 year old Idaho boys and girls, found mean serum cholesterol values of 133 and 144 mg. per 100 ml. for boys and girls, respectively. Except in this last instance, it would seem that the serum cholesterol values reported in this paper, both before or after orange juice supplementation, agree with the range of normalcy reported by other authors.

Ascorbic Acid

The boys and girls receiving the orange juice showed significant increases in their plasma ascorbic acid levels. Before supplementation, the mean plasma ascorbic acid level was 0.9 mg. per 100 ml.; and the level was the same both for the experimental and control groups. After receiving the orange juice, the experimental groups' ascorbic acid plasma level rose to a mean of 1.3 mg., while that of the controls remained the same.

At levels of ascorbic acid intake above 70 mg. daily blood plasma levels may vary between 0.6 and 1.0 mg. per 100 ml. plasma (9, 10, 27, 40). Mean levels may rise to as much as 1.4 mg. per 100 ml. at intakes of 100 to 1000 mg. daily (27). It already has been shown (Table I) that 10 ounces of orange juice contains 118 mg. of ascorbic acid so that it is not surprising to find increases of the magnitude which this experiment demonstrated.

Mack et al (29) fed orange juice to elementary school children and found significant increases in the plasma ascorbic acid levels. In one school, the mean plasma ascorbic acid level rose from 1.08 mg. per 100 ml. to 1.59 mg. after supplementation. A group of subjects from another school had a mean initial level of 1.09 mg.; and, after receiving their orange juice, the mean level increased to 1.77 mg. Certainly the increases in ascorbic acid levels reported

in the present paper confirm the increases found by Mack et al. They also are in agreement with the findings of Morse et al (35) even though their experimental design differed from the one reported in this paper in that they supplemented ascorbic acid *per se* and also their subjects were a group of older women.

Previously, the significance of maintaining ascorbic acid at the maximum plasma levels has been questioned. There is evidence from investigation on animals, however, that reproduction is improved and that other vital functions become more efficient with additions of ascorbic acid in the diet (42). In addition, Mack et al reported that they found improvements in the conditions of the gums of elementary school children given orange juice as a supplement at school. The subjects, after the ingestion of orange juice, also showed increases in growth and bone density, and elevations in hemoglobin. Of course, such improvements may not be due entirely to the increased intake of ascorbic acid supplied by the orange juice supplement, and the vitamins' consequential rise in plasma, but to various components in the orange juice itself, acting alone or in synergistic relationships. It could be postulated that the teen-agers in this, the more recent orange juice study, might demonstrate similar improvements; but at this time a report cannot be made about the outcome of such additional tests, since they still are undergoing

statistical treatment.

The initial blood analysis for ascorbic acid revealed that the mean level for this vitamin in the girls (regardless of group, experimental or control) was 0.91 mg. per 100 ml. of plasma. For the boys the initial mean was 0.94 mg., and for all the subjects (males and females) the mean level was 0.93 mg. These levels are in approximate agreement with those found by Odland and Ostle (36) and also those reported by Bring et al (15). Some workers (15, 17) have found that the mean levels for girls were significantly higher than those for the boys. This difference between the sexes, however, did not seem to exist in the present study.

Vitamin A

The vitamin A plasma levels for the experimental high school group showed a significant increase between Test 1 and Test 2. The mean level rose from 36.1 mcg. to 42.2 mcg. There was a slight increase in the vitamin A level of the controls, on the other hand, but the change was not significant.

The significant increase found in the experimental groups' vitamin A level represents a change not found by Mack et al (29) in a previous orange juice study. Conditions between the study of Mack et al and the one reported here, which she designed, did not differ greatly. In the present

study the initial plasma vitamin A level was considerably lower in the experimental subjects than that found in the control group which had a mean vitamin A level of 42.4 mcg. per 100 ml. plasma. At the end of the study, the level of the controls still was 0.2 mcg. higher than the level found in the experimental group. This was true despite the fact that the experimental group had increased their vitamin A levels significantly between tests. Thus, one might postulate that the vitamin A levels which are not up to blood "saturation" levels can be increased by the introduction of additional carotene, or more correctly supplementary orange juice to the diet. Merrow et al (32), however, reported that they could find no relation between plasma vitamin A and its dietary intake. Their report agrees with the conclusions made by Caster and Mickelsen (16) who also suggested that the level was more characteristic of the individual.

Beach et al (5) report a mean level of 42 mcg. per 100 ml. of plasma in 14-16 year old children. Somewhat lower levels were found by Bring et al (15), who reported mean levels of 31.5 and 34.5 mcg. for girls and boys, respectively. The findings reported in this study were comparable with the ones mentioned above. Our findings showed the girls to have a mean level of 36.3 mcg., and the boys showed a mean level of 40.5 mcg. per 100 ml. In a recent study conducted by Donald et al (19), slightly higher levels were shown. The

girls had an average level of 44.7 and the boys of 46.4 mcg. All of these investigators are in agreement with other authors (9, 17) who have reported that the concentration of blood plasma vitamin A of a large group of well-nourished children will lie, for the most part, between 30 and 50 mcg. per 100 ml. None of the investigators noted any major differences of vitamin A plasma levels between sexes.

The major portion of the subjects (52 per cent or over) had initial values which fell in the top range level of 35.5 mcg. or over. According to Bessey (8) "plasma vitamin A levels above 30-40 mcg. per 100 ml. can be taken as evidence that there is storage of this vitamin in the liver." This investigator also has said that "it seems apparent that, at vitamin A intake levels which lead to liver storage, factors other than intake become important in governing blood levels."

Carotene

Initially, the experimental high school group showed a mean carotene level of 0.101 mg. per 100 ml. plasma; and this increased during the study to 0.114 mg. per 100 ml. The controls started with a mean level of 0.086, or 0.015 mg. lower than the levels of the experimental group. By the final test, however, the levels of the control group had in-

creased to 0.118 mg. per 100 ml. Thus the mean carotene levels both in the experimental and the control groups showed significant increases.

A result of this sort makes it useless to postulate as to whether or not orange juice caused the rise in the experimental group. The plausible explanation for both the rises probably rests on the time of year when the testing took place. The first blood samples, before supplementation, were drawn in the fall of 1957 and the second test was taken in the spring of 1958. Therefore, it seems extremely probable that those foods rich in carotene were more available to consumers by the time the second test was made. In a survey made by Odland and Ostle (36) on Montana adolescents, it was reported that the carotene levels surveyed in summer were consistently higher than those surveyed in early fall. This would reflect a difference in food habits and the seasonal availability of certain foods. Bessey (8) also has reported that the carotene level will depend upon the green and yellow vegetable content of the diet. Merrow, Krause et al (32), in a study of nutrient intake and serum levels, noted that the dietary intake of carotene was reflected in the plasma level of carotene. The lower plasma levels demonstrated by the controls on the initial test may have allowed for the greater plasma carotene gain shown by them.

Some authors (9, 17) have reported that the plasma carotene concentration will lie, for the most part, between 0.075-0.200 mg. per 100 ml. plasma. These results have been confirmed by others (5, 15, 18, 36, 40); and they concur with those found in this study where the initial mean levels of carotene for the girls was found to be 0.095 mg. per 100 ml. and for the boys it was 0.101 mg. The average for both sexes was 0.093 mg. per 100 ml. plasma.

UNIVERSITY SUBJECTS

Cholesterol

Group I, the members of which received the orange juice supplement alone, was the only group to demonstrate any significant change in serum cholesterol levels between Test 1 and Test 2. The analysis of variance, when applied to this test, however, did not show that there were any real differences between the five groups, so that one can conclude that the differential supplements did not have any effect on the serum cholesterol levels.

The major portion of the girls in the study fell into Class 2 in cholesterol level, or the 150-199 mg. cholesterol per 100 ml. serum range. Even though all groups manifested a tendency to gain in cholesterol following supplementation, none of the gains except those obtained in Group I were sufficient to place the majority of the groups in a different class range.

Initially, the mean level for the 85 subjects was 181 mg. per 100 ml. serum. This value is almost the same as the average reported by Offenkrantz and Karshaw (37); and it does not differ greatly from the averages reported by other authors (15, 24). The value does differ to a large degree, however, from that reported by Odland and Ostle (36).

Ascorbic Acid

All of the supplementary groups had significant increases in their ascorbic acid measurements between Test I and Test 2. As with the serum cholesterol, an analysis of variance was made with no significant differences found between groups. When one observes the data closely, however, one can see that there does seem to be a tendency for those groups which received 150 mg. of ascorbic acid (II, III, IV, and V) to have mean gains slightly above the gain showed by Group I, which received only the orange juice. Even though this was true, it has been reported that, with the ingestion of high levels of ascorbic acid (100-1000 mg. per day), most of the larger doses are excreted and the blood level does not rise farther (8). Hence, with the ascorbic acid already present in the diet and that added by the orange juice supplementation (118 mg. per day), one would not expect extra ascorbic acid to increase the blood plasma levels of the vitamin.

At the time of the first test, the percentages of the subjects were scattered throughout the various classes or ranges. By the time of the second test, however, the major portion of the subjects in all experimental groups were to be found in Class 1, or the 1.10 mg.-and-over level. In fact, the smallest per cent to be found in this class was 75; and this figure increased up to 100 per cent in one group. The advantages of maintaining levels at this height already have been discussed previously in this paper.

The mean ascorbic acid level for all subjects was 0.78 mg. per 100 ml. plasma in the first test. This figure agrees with reports made by Clayton (17). On the other hand, it falls below the results of several other experimentalists (15, 29, 36).

Vitamin A

The subjects in Group I showed a significant increase in blood plasma vitamin A. Not only were they the only group to demonstrate such a rise from Test 1 to Test 2, but it happened despite the fact that Groups II and III received 25,000 I. U. of vitamin A daily (except Sundays) throughout the experiment. Also, this happened despite the fact that all groups finally received orange juice the last month of the experiment, so that one might be inclined to

believe that the gain in Group I might have been one of chance. This supposition is made for two other reasons: because there is no logical reason to suspect a greater increase in Group I than in any other group; and because the analysis of variance demonstrated that the differential rate of gain between the groups was not significant. Taking the group as a whole, however, orange juice had a tendency to increase, and the B-complex with ascorbic acid and Mellorine a tendency to decrease vitamin A levels.

Experiments conducted in which vitamin A was ingested either in oil or aqueous dispersion show that the level of the vitamin A rises to a maximum in about three hours for the latter and five hours for the former menstruum; and in both cases returns to normal in approximately 24 hours. Perhaps, after ingestion of certain levels of vitamin A, the plasma concentration does not increase, with the excess vitamin A being stored in the liver (42). Here again, as in the case of ascorbic acid, a lengthier discussion of the meanings of the levels of this vitamin in plasma have been presented previously in this thesis.

On the initial test, the majority of the subjects were in the high range (35.5 mcg. and over) of plasma vitamin A, and, regardless of supplements, this was the classification in which they tended to remain. One can take this as evidence that the largest percentage of the subjects had

storage of the vitamin in the liver, and that at these high levels, factors other than intake are responsible. Therefore, the blood levels are not a wholly reliable index of intake (8).

The mean level of all of the girls on the initial test was 46.9 mcg. per 100 ml. blood. This finding was for the most part in agreement with those of other workers.

Carotene

Again, Group I was the only experimental group which showed any significant change in the carotene levels between tests. Again, this group showed an increase. Also, this is the first instance in which the analysis of variance has shown a significant difference between the groups. Since Group I supplies the major portion of the difference, it should be pointed out that the initial level of this group was considerably lower than that in the other groups. Therefore, despite the fact that all groups received orange juice, Group I had further to go to reach plasma saturation and hence, could show a greater gain. As with vitamin A levels, orange juice had a tendency to increase, and the B-complex with ascorbic acid and Mellorine to decrease, plasma carotene levels.

As with the high school subjects, the major per cent of the girls in the different groups fell in Class 3 at the initial test, but for the most part, they rose to

Class 2, or the 0.190-0.150 mg. per 100 ml. level. Unlike the high school subjects, however, not all of the groups showed a rise in the carotene level. A comparison of the university subjects with the high school subjects showed that the Texas Woman's University subjects had much higher levels of carotene initially than did the latter. One possible explanation for this difference lies in the fact that most of the university girls eat in the school cafeteria, which is supervised by a trained dietitian, who sees to it that the foods containing the necessary nutrients are supplied at all times.

Initially, the mean carotene value for all girls was 0.142 mg. and this agrees with the normal values postulated by other authors (9, 17).

S U M M A R Y

Seventy-five high school subjects, including 32 females and 43 males, participated in a study conducted at the Nelda Childers Stark Laboratory for Human Nutrition Research at Texas Woman's University in Denton, Texas. A blood sample was drawn from each of them in the fall of 1957 and then a dietary supplement of 10 ounces of orange juice was given to them daily at school. This was continued until the spring of 1958 when the subjects were retested. In conjunction with this group, another 52 high school subjects, including 24 females and 28 males, were assayed in the same manner as described above, except they were treated as controls; that is, they were given no orange juice.

The blood was used to measure the serum cholesterol, plasma ascorbic acid, vitamin A, and carotene. These measurements were made on the initial test and again at the final testing period. Observations of the blood changes, if any, were made.

A significant decrease was noted in the serum cholesterol level from Test 1 to Test 2. The loss was found in both the experimental and the control groups. No explanation was offered for these results.

In the experimental group, a significant increase was found in the plasma ascorbic acid level from Test 1 to Test 2. The author felt this was due to the additional ascorbic acid supplied from the orange juice. There were no significant changes in the plasma ascorbic acid levels of the control group.

No significant changes were found in the levels of the vitamin A from the first test to the second test either in the experimental or control groups.

The carotene levels both in the experimental and control groups increased significantly. It was thought that in the control group this result might be due to the seasonal availability of those foods rich in carotene. A similar explanation could be offered for the increase in the experimental group, but probably it should be pointed out that the orange juice supplement could account for some of the rise found in the plasma levels (29).

In the part of the study involving university women, 85 subjects were divided into five supplementary groups. As in the case of the high school subjects, the university subjects were tested once in the fall, given their supplements, and retested in the spring.

Group I received a supplement of 10 ounces of orange juice.

Group II received a vitamin A capsule, Mellorine, and vitamin B-complex with ascorbic acid.

Group III received 10 ounces of orange juice, a vitamin A capsule, Mellorine, and vitamin B-complex with ascorbic acid.

Group IV received Mellorine and vitamin B-complex with ascorbic acid.

Group V received 10 ounces of orange juice, Mellorine, and vitamin B-complex with ascorbic acid.

During the last month of the study, Groups II and IV also were given 10 ounces of orange juice.

The results on the serum cholesterol levels showed that Group I had a significant increase from Test 1 to Test 2. There were no significant changes in the other groups even though all the groups showed slight gains. No conclusions were made about these increases.

The plasma ascorbic acid levels showed significant increases in all the groups. It was assumed that the ascorbic acid supplied by the orange juice, and also that supplied by the B-complex pill with ascorbic acid, or both, accounted for this rise.

Group I showed a significant increase in the plasma vitamin A level from Test 1 to Test 2. None of the other

groups showed significant changes. It was thought that the significant rise found in Group I occurred by chance.

The carotene levels from the first test to the second test showed a significant increase in Group I, but no significant changes were found in any of the other groups. It was thought that because the initial value found in Group I was considerably lower than the value found in the other groups on Test 1, then these subjects had further to go in order to reach plasma saturation, and hence could show a greater gain.

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