A STUDY OF THE BLOOD PLASMA LEVEL OF VITAMIN C IN A GROUP OF OLD PEOPLE

by
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CHAPTER I

Introduction

During recent years much research has been conducted but much remains to be accomplished to establish the optimal amount of vitamin C required by man. Scurvy as an outright deficiency disease has been studied and almost banished, but there are minor vitamin C deficiency effects on man that need closer study. The requirements of children and young adults have been widely investigated, but whether the requirement changes in old age has not been determined. A study of the vitamin C metabolism of the aged would, therefore, seem an interesting field of study.

Minot, Dodd, Keller, and Frank in a study of 540 children concluded that 1.0 milligram of vitamin C per 100 milliliters of blood serum was a satisfactory state of nutrition. Values as low as 0.3 mg./100 ml. indicate a low intake, but there is no clinical evidence of scurvy.[1]

At the present time an extensive study of the vitamin C metabolism of college men and women is being carried on by a group of universities in the Northwestern region. This Northwest Nutrition Cooperative Research group decided on the value of 0.8 mg. per 100 ml. for judging an adequate state of nutrition in accord with findings of Greenberg, Rinehart, and Phatak and with Neuweiler.[2] Farmer and Abt set 0.7 mg. per 100 ml. as prescurvy

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[1]A. S. Minot, Katherine Dodd, Margaret Keller, and Helen Frank, "A Survey of the State of Nutrition with Respect to Vitamin C in a Southern Clinic", Journal of Pediatrics, XVI (June, 1940), 717-728.

[2]Margaret L. Fincke and Virginia L. Landquist, "The Daily Intake of Ascorbic Acid Required to Maintain Adequate and Optimal Levels of This Vitamin in Blood Plasma", Journal of Nutrition, XXIII (May, 1942), 483-490.

level.[3] The higher the intake of vitamin C the higher the blood plasma level will be. Is this level of 0.8 mg. for young adults satisfactory for old people? Sherman states:

> "While as yet there have been no published researches into effects of different levels of vitamin C intake upon the complete life history, there seems no reason to doubt and much reason to believe that liberal levels of intake are permanently beneficial. In fact, the benefit may be greatest at the more advanced ages which have only recently received systematic attention of investigators. The decline in vitamin C content in human tissues after the age of 45 which was such a striking feature in the findings of King and co-workers probably indicates an increased rate of destruction or wastage of this vitamin in older people and that they should have vitamin rich food at frequent intervals."[4]

According to the 1940 census, there are nine million people in the United States over sixty-five years of age. Dublin predicts an enormous increase in the extension of life in the future; therefore, it seems in order to study the nutritional requirements of old people.[5] The present study was planned to observe the vitamin C level of blood plasma in a group of old people sixty years of age and over living on the regular diets of an institution. Blood samples were taken from the finger of all persons within the age group in the institution who were willing to cooperate in the study. The micro method for the analysis of blood plasma

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[3]Chester J. Farmer and Arthur F. Abt, "Titration of Plasma Ascorbic Acid as a Test for Latent Avitaminosis", Milbank Memorial Fund: Nutrition: The New Diagnostic Methods, (1938), p. 114-137.

[4]Henry C. Sherman, The Science of Nutrition, (New York: Columbia University Press, 1943), p. 53.

[5]Louis I. Dublin, Medical Problems of Old Age, (New York: Metropolitan Insurance Co.)

of Farmer and Abt[6] was used and the ascorbic acid content of the diet for the day preceeding the sampling was calculated using the nutritive value tables of Taylor.[7] A test dose of varying amounts of vitamin C was given to a selected group of old people to observe the response to an increased intake of the vitamin.

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[6]Chester J. Farmer and Arthur F. Abt, "Ascorbic Acid Content of Blood", Society of Experimental Biology and Medical Proceedings, XXXII (1935), 1625.

[7]Clara Mae Taylor, Food Values in Shares and Weights, (New York: The MacMillan Company, 1942).

CHAPTER II

Review of the Literature

There have been numerous reports of vitamin C studies with young children and with college men and women. Little attention has been paid to the vitamin C requirements or metabolism in the aged. Some of the studies on the vitamin C requirement dealing with children and young adults will be cited.

In a southern pediatric clinic Minot, Dodd, Keller, and Frank used the blood plasma test to determine the vitamin C status of 540 children. The results were evaluated by use of standards of 0.7 mg. vitamin C and over per 100 ml. of plasma as indicative of a satisfactory state of nutrition with respect to vitamin C. Values of 0.3 to 0.7 mg. vitamin C per 100 ml. indicated restricted intake which may or may not be associated with serious undersaturation of the tissues and values under 0.3 mg. indicated serious deficiency of vitamin C.[8]

In a study of infants and children, Abt and Farmer observed no correlation between age and vitamin C level of the blood. There was a direct variation of the blood plasma level with the vitamin C intake. Children with active scurvy were found to have the lowest blood plasma levels. The plasma values showed a rapid rise during the recovery period and were more informative of the state of vitamin C nutrition than single or twenty-four hour urinary values.[9]

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[8]A. S. Minot, Katherine Dodd, Margaret Keller, and Helen Frank, "A Survey of the State of Nutrition with Respect to Vitamin C in a Southern Pediatric Clinic", Journal of Pediatrics, XVI (June, 1940), 717-728.

[9]Arthur F. Abt and Chester J. Farmer, "Cevitamic Acid Content of Blood Plasma", American Journal of Diseases of Children, LIV (September, 1937), 682.

Levcowick and Batchelder in a study of the vitamin C metabolism of forty-five young women concluded that 50 mg. was a minimum requirement of vitamin C for a moderately active woman, while a dietary standard of 75 mg. daily was satisfactory.[10] The Northwest Nutrition Cooperative Group chose 0.8 mg. of ascorbic acid per 100 ml. as a criterion for judging an adequate state of nutrition. Neuweiler considered 0.8 mg. as a "good normal" value without tissue saturation as cited by Levcowick and Batchelder. They quoted Greenberg, Rinehart, and Phatak as accepting 0.7 - 0.9 mg. as adequate but not optimal.[11] Farmer and Abt chose 0.7 mg. as the prescurvy level.[12] Fincke and Landquist found the blood plasma levels paralleled the vitamin C intake more closely than did the response to the test dose. It was their opinion that plasma levels represented a better criterion than those of excretion for judging saturation after a test dose. They stated that there is no relation between body weight and the ascorbic acid required to maintain saturation levels in the blood.[13]

Brown and others grouped the blood plasma levels according to the classification suggested by Neuweiler. They considered below 0.4 mg. as definitely scorbutic, although they realized that no symptoms of scurvy

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[10] Tatiana Levcowick and E. L. Batchelder, "Ascorbic Acid Excretion at Known Levels of Intake as Related to Capillary Resistance, Dietary Estimates, and Human Requirements", Journal of Nutrition, XXIII (April, 1942), 399-408.

[11] Ibid., p. 399.

[12] Abt and Farmer, op. cit., p. 114.

[13] Margaret L. Fincke and Virginia L. Landquist, "The Daily Intake of Ascorbic Acid Required to Maintain Adequate and Optimal Levels of This Vitamin in Blood Plasma", Journal of Nutrition, XXIII (May, 1942), 483-490.

were observed; 0.4 mg. to 0.79 mg. as representing a deficiency of vitamin C; 0.8 mg. to 0.99 mg. as a good normal value without saturation; and 1.0 mg. or more as saturation.[14]

Studies such as those of Todhunter and Rollins revealed that after test doses of 400 mg. the blood level was not raised, indicating saturation. When the intake of ascorbic acid was lowered each day for six days, the level was brought back to 1.4 mg. per 100 ml. There is a question whether the level should be maintained at 1.4 mg. for optimum health. More than 120 mg. of vitamin C daily was required by each subject to maintain this level.[15]

Some possible factors affecting the vitamin C metabolism in an individual include amount of exercise, body size, and presence of an infection or wound. Brown and others cited that exercise might increase the requirement of the individual while height, weight, and age seemed to have no effect.[16] Belser, Hauck, and Storvick limited the exercise of subjects being studied because of the effect of exercise on vitamin C metabolism.[17] That exercise increased the requirement of vitamin C was also reported by Goldsmith and others. They considered the human requirement

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[14]Almeda P. Brown, Margaret L. Fincke, Jessie E. Richardson, E. Neige Todhunter, and Ella Woods, "Ascorbic Acid Nutrition of Some College Students", Journal of Nutrition, XXV (May, 1943), 411-426.

[15]E. Neige Todhunter and R. C. Rollins, "Amount of Ascorbic Acid Required to Maintain Tissue Saturation in Normal Adults", Journal of Nutrition, XIX (March, 1940), 263-270.

[16]Brown, op. cit., p. 411.

[17]W. B. Belser, H. M. Hauck and C. A. Storvick, "A Study of the Ascorbic Acid Intake Required to Maintain Tissue Saturation in Normal Adults", Journal of Nutrition, XVII (June, 1939), 513-526.

of vitamin C to be related to body weight, but Ralli and her co-workers did not agree.[18] Todhunter found some evidence that fatigue and perspiration cause a lower vitamin C blood level.[19] Esselen and Fuller stated that scurvy may sometimes be caused by destruction of vitamin C by bacteria in the upper part of the intestinal tract before the vitamin can be absorbed.[20] A relation of vitamin C intake to the healing of wounds was suggested by work of Lund and Crandon.[21]

In the comparatively few studies dealing with older persons, low plasma levels of vitamin have been frequently encountered, especially in senile individuals. Stotz, Shinner, and Chittick in a study of six senile patients found that five of them showed a level of 0.35 - 0.70 mg. per 100 ml. which was considered to indicate deficiency. The diet was adequate in vitamin C so that the deficiency was not due to dietary habits nor to faulty intestinal absorption as oral ingestion of a test dose demonstrated. Further investigation to discover the cause was planned.[22] Is this

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[18] Grace A. Goldsmith, Adolph T. Ogaard, and Donald F. Gowe, "Estimation of the Ascorbic Acid Requirement of Ambulatory Patients," Archives of Internal Medicine, LXVII (March, 1941), 590-608.

[19] E. Neige Todhunter, "The Newer Knowledge of Vitamin C in Health and Disease", Journal of American Dietetic Association, XVI (January, 1940), 1-11.

[20] W. B. Esselen, Jr. and J. E. Fuller, "Oxidation of Ascorbic Acid as Influenced by Intestinal Bacteria," Journal of Bacteriology, XXXVII (May, 1939), 501-521.

[21] Charles C. Lund and John H. Crandon, "Human Experimental Scurvy", Journal of American Medical Association, CXVI (February 22, 1941), 663-668.

[22] E. Stotz, B. M. Shinner, and R. A. Chittick, "The Oral Ascorbic Acid Tolerance Test and Its Application to Senile and Schizophrenic Patients", Journal of Laboratory Clinic Medicine, XXVII (1942), 518.

deficiency associated with the pathology of senility rather than with old age itself? In 14 other patients 65-90 years, plasma values of over 0.70 mg. were found. The relationship of vitamin C retention to arteriosclerosis is being studied by Rafsky and Newman.[23]

Yavorsky, Almeden, and King investigated the amount of ascorbic acid in human organs at different ages. They found that with ageing the organs become poorer in this vitamin.[24] Stephenson and co-workers quoted Remp, Rosen, Ziegler, and Cameron; Plaut and Bulow; and Gander and Neiderberger as being able to increase this low level by oral administration of ascorbic acid which appears to exclude the possibility that this condition can be caused by decreased absorption of the vitamin from the intestinal canal of senile persons. A probable cause of the low vitamin level would be deficiency of vitamin C in the diet of old people.[25] However, it was also cited by Stephenson that the clinical observations of Plaut and Bulow and of Monauni indicate that the decreased amount of vitamin C in older persons can not be explained merely by the low content of this vitamin in the diet, because the difference between young and old persons is present even when both groups were receiving the same hospital diet.[26] Of course there is the possibility that a higher level of ascorbic

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[23]H. A. Rafsky and B. Newman, "Vitamin C Studies in The Aged", American Journal of Medical Science, CCI (1941), 749-756.

[24]Martin Yavorsky, Phillip Almeden, and C. G. King, "The Vitamin C Content of Human Tissue", Journal of Biological Chemistry, CVI (September, 1934), 525-529.

[25]W. Stephenson, C. Penton, and V. Korenchevsky, "Some Effects of Vitamin B and C on Senile Patients", Reprinted from the British Medical Journal, II (December 13, 1941), 839.

[26]Ibid, p. 839.

acid intake was needed by the older persons than the younger ones. It has been suggested that in senile persons, there is a decrease in intensity of those metabolic processes in which vitamin C is taking part. Gander and Niederberger according to Stephenson, found that treatment of senile persons with adequate doses of ascorbic acid was followed in some cases by improvement of the general health, increased vitality, better sleep, disappearance of rheumatic pains, and a favorable effect on pneumonia of senile patients. Schroeder onsiders 50 mg. of ascorbic acid to be the minimum daily dose for human beings.[27]

A great deal of advancement in nutrition has been made by experiments with animals and basic knowledge gained in that manner has been applied to man. It is, therefore, of interest as this study is begun to cite the work of Bessey and King with tissues of rats, rabbits, and chickens. They found a distinct tendency for the tissues of young rapidly growing animals to be higher in vitamin C content than those of older animals on a similar diet.[28] Whether or not this applies to humans will require further study with old people to determine their vitamin C needs.

Much work has been done with children and young adults and 75 mg. of vitamin C daily has been recommended by the National Research Council, yet the amount needed for optimal health at various ages has not been determined.[29] Does this requirement change with age? This question needs investigation.

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[27] Ibid, p. 839.

[28] O. A. Bessey and C. G. King, "The Distribution of Vitamin C in Plant and Animal Tissues and Its Determination", Journal of Biological Chemistry, CIII (December, 1933), 687-698.

[29] Taylor, op. cit., 54.

The foregoing references have been cited as representative of work which has been done in investigating the vitamin C metabolism of people. The method most widely accepted was used for determining the amount of vitamin C in the present study of old people. Approved technics were employed in collecting the blood, in running the tests, and in tabulating the data in order to obtain sound facts regarding the vitamin C status of this group of old people and their response to a test dose.

CHAPTER III

Procedure

Time, Place, and Subjects.

This study was conducted during March and April, 1944. A survey was made to ascertain the vitamin C plasma level of a group of old people living at the Guilford County Home in Greensboro, North Carolina. Then, to three smaller groups of these people test doses of concentrated vitamin C were given and samples of blood taken after three hours and after twenty-four hours.

There were sixteen men and ten women whose ages ranged from sixty to eighty-eight years. Table 1 shows the distribution of ages represented. While only a few seemed to be in good health, none of them were bed ridden. Two subjects had mild cases of diabetes mellitus and two had suffered a stroke of paralysis and were confined to wheel chairs.

Table 1. Distribution of Age

Age	Number of men	Number of women
60-64	6	2
65-69	4	2
70-74	2	2
74-79	3	4
80-84	0	0
85-89	1	0

Diet.

All of the subjects except the diabetics ate the regular diet of the institution. Personal likes and dislikes further limited the foods actually eaten. The diet did not furnish regularly an adequate amount of vitamin C according to the recommended daily allowance due to the season of the year and to economic conditions. Oranges were available only to those sick in bed or to those who could afford to buy them.

Several people bought eggs and butter to supplement their diet, but the majority of them did not have the money to spend. A record was kept of the food eaten on the day previous to the sampling for each individual. The blood samples were taken after breakfast at which no food containing an appreciable quantity of vitamin C was consumed.

Experimental.

After the fasting vitamin C plasma levels had been determined, ascorbic acid was given to three groups of five people each. One group was given a dose of 75mg., another group 150 mg., and the last one 300 mg. immediately after breakfast. At the end of three hours a sample of blood was taken to ascertain the amount of the test dose that had been absorbed into the blood stream. Twenty-four hours later another sample was taken to find out how much vitamin C remained in the blood plasma.

Blood Plasma Analysis.

The micro method of Farmer and Abt was used for the determination of vitamin C in blood plasma. Lithium oxalate was placed in small vials used for collecting fourteen to fifteen drops of blood. These were placed in an ice chamber immediately upon mixing the oxalate with the blood. The samples were then taken directly to the college nutrition laboratory for the analysis. The blood was centrifuged to separate the red cells from the blood plasma, and the latter pipetted out. The plasma was then diluted with water, deproteinized with metaphosphoric acid, and again centrifuged. A clear plasma was obtained this time and after being pipetted into a white porcelain testing plate, it was titrated with a dilute dye solution of sodium 2,6 dichlorophenol indophenol. The dye solution had been standardized previously against a solution of vitamin C. The very first

appearance of a faint pink color was taken as the end point. Computations were then made to determine the amount of vitamin C present per 100 milliliters of orginal blood.

CHAPTER IV

Discussion of Results

Average Daily Intake.

In making the dietary study of the vitamin C intake of the subjects, the amount of vitamin C in the diet of the institution was computed. The principal sources of vitamin C in the menus were not oranges, other citrus fruits, and tomatoes because of the prohibitive prices of these items, but were raw milk, cooked potatoes, raw cabbage, cooked green beans, cooked turnip greens, and vegetable soup. Each subject except No. 19 and No. 25 drank a pint of raw milk daily. A majority of them ate some turnip greens, slaw, and green beans. Potatoes were a favorite with all subjects. Bessey and White, however, stated that the amount of ascorbic acid consumed daily from sources other than citrus fruits and tomatoes; that is, from other fruits and vegetables were insufficient to influence the blood plasma values in their study.[30]

As the blood samples were taken, the subjects were asked which foods on the menus of the previous day they had eaten. As a rule the vitamin C content of the diet did not vary much, but occasionally it did for individuals on different days. Subjects 11, 13, and 14 had a high intake on days of the study, but this did not represent their normal consumption of vitamin C foods. When further investigation was made of their dietary habits, it was found that their regular intake of vitamin C was consistently low. It would appear that a day or two of irregularly high

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[30] Otto A. Bessey and Ruth L. White, "Ascorbic Acid Requirements of Children", Journal of Nutrition, XXIII (February, 1942), 195-204.

vitamin C consumption was not sufficient to affect the fasting plasma level. The average intake of vitamin C was 29 mg. and the range of intake was from 2 mg. to 85 mg. Table 2 shows the difference in the intake of

Table 2. Vitamin C Intake and Plasma Levels

Men				Women			
Subject	Age	Vitamin C Intake	Fasting Plasma Level	Subject	Age	Vitamin C Intake	Fasting Plasma Level
		Mg.	Mg.			Mg.	Mg.
1	78	44	0.07	17	67	27	0
2	69	14	0	18	65	27	0.07
3	62	40	0.07	19	75	0	0
4	64	40	0.14	20	76	33	0
5	68	0	0.07	21	62	27	0
6	75	30	0.28	22	78	31	0
7	68	14	0.28	23	70	17	0
8	62	0	0.07	24	62	33	0
9	70	30	0.28	25	75	17	0.14
10	60	30	0	26	71	22	0.14
11	68	85	0.14				
12	79	34	0.07				
13	61	65	0				
14	60	66	0.21				
15	73	14	0.28				
16	88	32	0.21				
Average		36				23	

the men and women. The men had better appetites and had fewer dislikes for vegetables. The men had an average intake of 36 mg. in comparison to a 23 mg. average for the women. Fincke and Landquist found that the daily intake necessary to maintain a plasma level of 0.8 mg. was approximately 1.1 mg. per kilogram of body weight.[31] By this criterion the

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[31]Fincke and Landquist, op. cit., p. 483.

intake of these subjects was quite deficient.

Initial Plasma Values.

The plasma value of the blood was found to be very low which was to be expected as the dietary intake definitely influences the vitamin C level of the plasma. Table 2 above gives the values as found in these subjects. In some the intake was higher than in others, but no appreciable amount of vitamin C was found for even the highest plasma values were definitely low. The variation in plasma value in different individuals is not very great. The intake of vitamin C appears to vary to a greater extent in the three days of study than is usually the rule as the vitamin C content is low in the institution diet in the winter-spring months.

The plasma level of the women was fairly consistent with the dietary intake as shown in Table 3. The vitamin C intake of the men seemed more varied as the menus served the day prior to taking their plasma value were higher in vitamin C than the average day.

75 Milligram Test Dose.

To a group of five old people, three men and two women, a test dose of 75 mg. of vitamin C was given after a vitamin C-free breakfast. After three hours a sample of the blood was taken to determine the amount of vitamin C absorbed. Then after twenty-four hours another sampling was taken to determine the fasting level following the test dose. The results are shown by Graph 1 and by Table 3.

Two of the men did not show any response to the test dose in three hours, but did give an increased plasma value in twenty-four hours. It is likely that their absorption is slow. The women showed an increase

in vitamin C value in three hours and a higher value in twenty-four hours. All subjects had an increased plasma value in twenty-four hours as a response to the supplement of the recommended daily allowance.

Table 3. Plasma Levels After 75 Mg. Test Dose

Subjects	Dietary Intake	Fasting Plasma Level	Dietary Intake	Test Dose	Plasma Level 3hrs.	Dietary Intake	Plasma Level 24hrs.
	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.
7	14	0.28	10	75	0	22	0.48
11	85	0.14	57	75	0	36	0.48
5	0	0.07	17	75	0.72	10	0.48
17	27	0	17	75	0.18	38	0.24
22	31	0	52	75	0.06	26	0.30

150 Milligram Test Dose.

To observe how much greater response would be obtained with twice the recommended daily allowance, a 150 mg. test dose was given to another group of five old people. Here again the men did not show as rapid response as the women. It was impossible to secure a twenty-four hour sample from subject 15. The results of this group are shown in Table 4 and also by Graph 1.

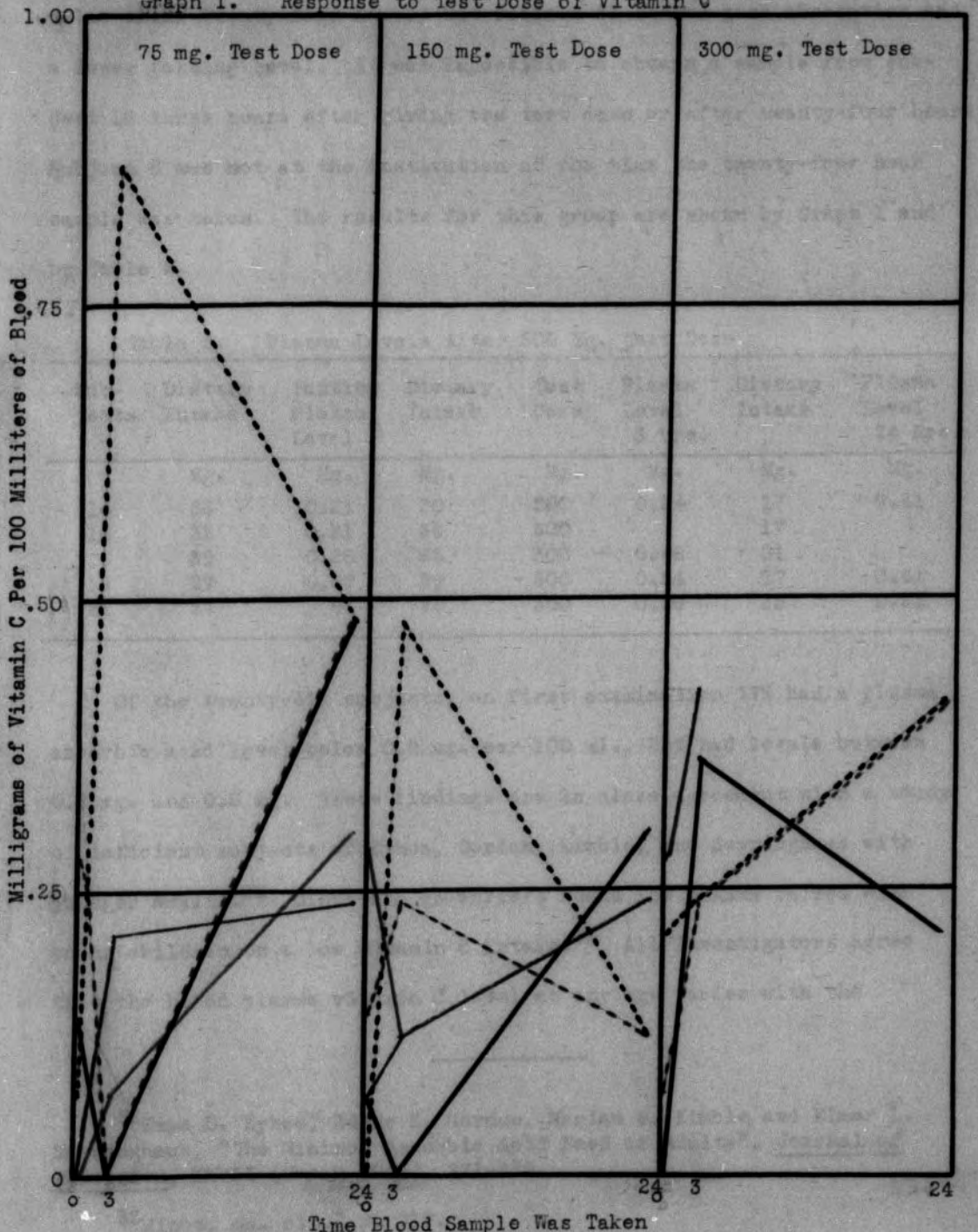
Table 4. Plasma Levels After 150 Mg. Test Dose

Subjects	Dietary Intake	Fasting Plasma Level	Dietary Intake	Test Dose	Plasma Level 3hrs.	Dietary Intake	Plasma Level 24hrs.
	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.
8	0	0.07	32	150	0	10	0.30
15	14	0.28	24	150	0.12	18	
1	44	0.07	24	150	0.12	18	0.24
25	17	0.14	22	150	0.24	32	0.12
19	0	0	6	150	0.48	0	0.12

300 Milligram Test Dose.

Only when a test dose of 300 mg. of vitamin C was given in powder form did the men show an increase in plasma value in three hours. The women

Graph 1. Response to Test Dose of Vitamin C



Subject

--- 5,19,14 ---- 7,25,18 — 22,16

— 11,8,21 — 17,1,6

(Incomplete record for 16 and 6)

showed a more rapid response. Subject 18 continued to have a higher value after twenty-four hours, but subject 21 showed good absorption and a lower fasting level. It was impossible to obtain a sample from subject 16 three hours after giving the test dose or after twenty-four hours. Subject 6 was not at the institution at the time the twenty-four hour sample was taken. The results for this group are shown by Graph 1 and by Table 5.

Table 5. Plasma Levels After 300 Mg. Test Dose

Subjects	Dietary Intake	Fasting Plasma Level	Dietary Intake	Test Dose	Plasma Level 3 hrs.	Dietary Intake	Plasma Level 24 hrs.
	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.
14	66	0.21	30	300	0.24	17	0.41
16	32	0.21	35	300		17	
6	30	0.28	65	300	0.42	21	
18	27	0.07	30	300	0.24	17	0.41
21	27	0	20	300	0.36	13	0.21

Of the twenty-six subjects, on first examination 77% had a plasma ascorbic acid level below 0.2 mg. per 100 ml., 23% had levels between 0.2 mg. and 0.6 mg. These findings are in close agreement with a study of deficient subjects of Kyhos, Gordon, Kimble, and Sevringhaus with younger adults.[32] Minot and co-workers found low plasma values with young children on a low vitamin C intake.[33] All investigators agree that the blood plasma vitamin C level at any age varies with the

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[32]Emma D. Kyhos, Edgar S. Gordon, Marian S. Kimble, and Elmer L. Sevringhaus, "The Minimum Ascorbic Acid Need of Adults", Journal of Nutrition, XXVII (March 1944), 271-285.

[33]Minot, op. cit., p. 717.

vitamin C intake and in order to raise the plasma level to a higher level the diet must be improved or supplements given.

The question arises whether the plasma level of this older group can be raised to the higher levels considered desirable for younger groups. Varying amounts of supplementary vitamin C were given to three groups of old people. The amount of the dose was respectively 75 mg., 150 mg., and 300 mg.

Investigators have found that maximum concentration of vitamin C is reached following a test dose in about three hours. Goldsmith and Ellinger gave their subjects a test dose of 600 mg. and took blood samples at the end of three hours, six hours, and twenty-four hours. They found the highest vitamin C concentration was reached in three hours with a slight fall after six hours.[34] Taylor, Chase, and Faulkner gave 1 gm. of vitamin C and found a rise which reached a peak in one hour and subsided in five hours. In one case of scurvy the rise was delayed, reaching the peak in three hours.[35] When Goldsmith and Ellinger worked with a group of fourteen deficient persons they secured the maximum concentration of vitamin C in the blood in one to three hours after the test dose was given except in three cases. It was thought the difficulty in these cases was due to slow absorption from the gastro-intestinal tract.[36]

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[34]Grace A. Goldsmith and George F. Ellinger, "Ascorbic Acid in Blood and Urine After Oral Administration of a Test Dose of Vitamin C", Archives of Internal Medicine, LXIII (March, 1939), 531-546.

[35]Frances Henry L. Taylor, Dorrance Chase, and James Morison Faulkner, "The Estimation of Reduced Ascorbic Acid in Blood Serum and Plasma", Biochemistry Journal, XXX (July, 1936), 1119-1125.

[36]Goldsmith, op. cit., p. 531.

In light of the findings of the other investigators cited, three-hour and twenty-four hour samples were taken in this study. It seemed impractical in every respect to take more that one sample of blood a day from these old people so the six-hour sample often taken by others was omitted. There was a wide variation in the response observed on each of the test doses as shown in Graph 1. Some individuals showed a rise in plasma ascorbic acid over the fasting level in three hours while others did not. Some individuals showed a higher level in the twenty-four hour fasting value than in three hours. In all but one case there was a slight increase in the fasting level following the dose. But in no case did this fasting level exceed 0.50 mg. per 100 ml. This level is considered very low by all investigators. Stotz, Shinner, and Chittick found that in individuals very deficient in vitamin C they did not get a marked rise in the plasma level following a test dose. They advanced the idea that absorption may be normal but in subjects with depleted tissues the rate of utilization may be so rapid that the plasma level does not show much or any rise in vitamin C content.[37] The reason that a marked rise in plasma vitamin C was not found in three hours in most individuals in this study might have been either slow absorption or rapid utilization.

Since the dietary intakes of vitamin C of the individuals in this study were so low, the low plasma ascorbic acid values found can be explained on this basis alone. As previously stated, regardless of age, low vitamin C intakes result in low plasma levels of vitamin C. Therefore, it was not possible to determine any difference in vitamin C levels due to age as

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[37]Stotz, op, cit., p. 518.

such unless the response to added supplies of vitamin C was observed.

It was decided to determine whether an adequate intake of vitamin C for a period of two weeks would have any effect on the plasma levels of these old people. A group of four volunteers was given 75 mg. of ascorbic acid daily, the recommended daily allowance set by the National Research Council for adults. This amount was selected for the diet at best probably would not exceed 75 mg. daily. Then, the response received from the various test doses was about as good for the lowest dose as for the highest. The 75 mg. given was in addition to the small regular supply of vitamin C in the subjects diets, 17 mg. to 32 mg. daily for the period of study.

In the experiments of Dodds and MacLeod with college students, all subjects showed an increase in plasma ascorbic acid values in two weeks on an 82 to 85 mg. intake, comparable to the total intake in the present study. Of course their subjects had previously had an adequate intake of vitamin C, but had been on a deficient intake (32 - 35 mg.) for the two weeks just prior to the study.[38]

Kyhos and associates working with prisoners who had been on an adequate intake of vitamin C for some time previous to the study observed a rise in vitamin C plasma levels when 50 mg. of vitamin C was given for two weeks.[39] It would appear, therefore, that the lack of response

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[38]Mary L. Dodds and Florence L. MacLeod, "Blood Plasma Ascorbic Acid Values Resulting Normally Encountered Intakes of this Vitamin and Indicated Human Requirements", Journal of Nutrition, XXVII (January, 1944), 77-87.

[39]Kyhos, Gordon, Kimble, and Sevringhaus, op. cit., p. 271.

observed in this study to a daily supplement of 75 mg. of vitamin C for two weeks might be characteristic of this older group. Table 6 shows the response received during the supplementary period in this study. Variations were found in each subject from day to day as well as in the group receiving the same supplement. These small variations are not indicative as they might have occurred had they not received any supplement of vitamin C. Dodds and MacLeod stated that an increase in intake did not immediately affect the fasting blood plasma ascorbic acid. Frequently the plasma value on the day following increased intake was decreased and was in general not typical of the intake, but of the fluctuation. They quoted Holmes, Cullen and Nelson as suggesting that an average of series of daily values gave a better estimate of the plasma ascorbic acid than single values taken at intervals.[40] The average fasting blood plasma level in this study was between 10 mg. and 21 mg. which is within the low deficiency level. These results may indicate that the subjects needed supplements for a longer period or needed a larger dose of vitamin C to bring their fasting blood plasma level up to a normal level. This may mean that older people need more vitamin C than younger adults. At least, it would be

Table 6. Response to Supplement of Vitamin C for Two Weeks

Subjects	Average Vit. C Intake	Fasting Plasma Level	Average Vit. C Intake	Fasting Plasma Level	Average Vit. C Intake	Fasting Plasma Level	Average Vit. C Intake	Fasting Plasma Level
	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.
1	17	0.30	24	0.06	19	0.30	17	0.18
14	23	0.06	19	0.12				
8	20	0.18	23	0	28	0.24	29	0.18
11	20	0	24	0.12	32	0.18	29	0.12

[40] Dodds and MacLeod, op. cit., p. 77.

interesting to continue the study to see how long it would take a 75 mg. supplement or better an adequate diet to raise the plasma level to 0.8 mg., a standard accepted by other investigators as desirable for good health.

Physical Condition:

The group that volunteered to take the 75 mg. supplement for two weeks was asked questions about their physical condition which might indicate vitamin C deficiency or scurvy symptoms. Three had lost their teeth, two due to pyorrhea, and one due to dental caries. They each acknowledged sore gums although subject 8 said his gums were "not so bad." Subject 1 bruises very easily while the other two reported that they seldom bruise. All complained of weakness and soreness in joints, especially in damp weather with a tendency to rheumatic pain for subjects 1 and 11. The latter does not have colds while the others have frequent colds. The general resistance of these subjects seemed to be low although they appeared well. Each one seemed to think the vitamin C did them good and offered that information of their own accord.

CHAPTER V

Summary, Conclusions, and Recommendations

The purpose of this study was to observe the vitamin C in the blood plasma of a group of old people. A survey was made of the plasma ascorbic acid level of all those over sixty years old living at the Guilford County Home, Greensboro, North Carolina who were willing to cooperate. Some did not bleed very freely so that their blood did not give accurate results due to hemolysis. These results were discarded so that the final number studied was 26 subjects.

In the initial sample of the blood of these subjects receiving the same diet a variation in plasma ascorbic acid levels was observed. The women did not show any or showed very little vitamin C in their blood plasma. All of the men had low levels, but they did show some plasma ascorbic acid. All subjects were in the deficiency level noted by other investigators.

It was then planned to give groups of these old people varying amounts of vitamin C as a test dose to see what response in plasma ascorbic acid would be observed. It was believed that a rise would occur for in general the plasma vitamin C varies with the intake. The test dose was given immediately after breakfast. Most authorities agree that the greatest absorption and rise in the blood would occur in three hours; therefore, a sample of the blood was taken at that time. A six-hour sample which some investigators think gives another view of the plasma was omitted in this study as most of the subjects objected to giving more than one sample of blood a day. In some of the subjects a rise in vitamin C content of the blood plasma was observed while others gave an even lower

reading. It is believed by some that this fluctuation is due to rapid utilization of the vitamin C or it may be due to slow absorption. The fasting plasma level taken in twenty-four hours gave a higher reading in the majority of cases which lead to the question could the blood plasma levels of these old people be raised to the higher level of 0.8 mg. an accepted standard of vitamin C in the blood plasma.

Therefore, four subjects were further observed for two weeks. Since the results obtained with 75 mg. test dose were as high as those on larger doses, 75 mg. the recommended daily allowance was given as supplement to their low vitamin C (17 - 32 mg.) diet. Twice a week blood samples were taken and variations in the plasma were observed both in the individual and within the group. Subject 14 was irregular in taking supplement and was not present when the last two samples were taken. The plasma levels of the three remaining subjects were still within the deficiency level at the end of the study.

This study has demonstrated:

1. That on a low vitamin C intake a low plasma value was observed in old people as it was in other groups.
2. That the recommended daily allowance, 75 mg. of vitamin C, was not sufficient to raise the plasma ascorbic level in two weeks.
3. That the plasma level was not raised to a higher degree when larger test doses of vitamin C were given than when 75 mg. was given.
4. That there were variations in an individual from day to day.

This study has suggested:

1. That the utilization of vitamin C in old people may be different from that of other groups.

2. That possible factors making the difference in old people are:

    1. Absorption may be so slow that the body does not get the full benefit of the vitamin intake.
    2. The body may utilize the vitamin so rapidly that it does not remain in the blood.
    3. Old people may need more vitamin C than young adults or children.

3. That some symptoms of vitamin C deficiency may appear in old people on low intake (17 mg. to 32 mg.) found in this study as the following indications were observed in some subjects.

    1. Sore gums
    2. Pyorrhea
    3. Dental Caries
    4. Tendency to bruise easily
    5. Soreness in joints
    6. Tendency to rheumatic pain
    7. Weakness
    8. Colds

The writer wishes to offer these recommendations:

1. That further study of the vitamin C utilization in regard to old people be instigated.

    1. Some interesting investigations might be:
        a. to continue the 75 mg. supplement for a longer period of time to determine how long it would take to raise the plasma ascorbic acid to a safe level.
        b. to give large doses of vitamin C supplement to raise the plasma ascorbic acid level.

c. to give foods of known ascorbic acid value to determine how much is necessary to raise the level of plasma ascorbic acid.

2. That the diet of those living in an institution contain more sources of vitamin C as oranges, tomatoes, and cabbage.

a. Additional vitamin C may be obtained by including turnips, sprouting seed, carrots, lettuce, asparagus, peppers, and the various members of the "greens" family.

b. The above foods served fresh and raw as often as possible.

3. The amount of vitamin C in these foods may be better preserved by shorter cooking time.

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APPENDIX

Micro Test For Ascorbic Acid in Plasma

(Farmer and Abt)

Reagents.

1. The Dye. Sodium - 2, 6 dichlorophenol indophenol in powder form is used. The powder is extracted with boiling water to make a stock solution, which must then be diluted and standardized against pure crystalline ascorbic acid. The dilute stock solution is further diluted (1:2) for titrating the deproteinized plasma.

2. Metaphosphoric Acid (HPO_3). This material is used as the deproteinizing agent for the plasma in a strength of 5%. It is also used as a solvent for ascorbic acid in the standardization of the stock dye in a strength of 2.5%. In solutions of HPO_3, the dye assumes a pink color instead of the deep blue seen in neutral solutions.

3. Ascorbic Acid. Any pure crystalline ascorbic acid may be used to standardize the dye.

4. Lithium Oxalate. This material is preferred as an anticoagulant to other oxalates. A 1 - mm. pile of the powder on the end of a toothpick is placed in the micro-blood bottle.

Apparatus.

In addition to the ordinary glassware which is a part of the equipment of any chemical laboratory, there is required a microburette, a chemical balance and a centrifuge. Special apparatus which is required consists of the micro-blood bottles and a special microburette reading directly to 0.002 cc.

Procedure.

The first step in the procedure is the standardization of the dye. After diluting the stock dye 1:10 with water, this is titrated against an

accurately made solution of ascorbic acid in 2.5% HPo_3. The reading is taken when the first faint pink color is produced. The calculations are:

(Ml. dye to ascorbic acid - Ml. dye to 2.5% HPo_3) = (2 x mg. of vit. C)

or

$$\frac{\text{(2 x mg. of vit. C}}{\text{(Ml. dye to ascorbic acid - Ml. dye to 2.5\% } HPo_3\text{)}} = \text{standardization factor}$$

The next step is to obtain the blood, centrifuge out the cellular elements, deproteinize, and titrate. The reading of the HPo_3 when the first faint pink color appears is substracted from the reading of the plasma. The remainder is used for the final calculation as the plasma reading.

Plasma reading x standardization factor x 1000 = mg. of vit. C per 100 ml.

Interpretation.

On an adequate diet the normal range of ascorbic acid in the blood by this method has been established as 0.7 milligrams or above per 100 milliliters. Values which are below 0.5 milligrams per 100 milliliters are considered to indicate a marked insufficiency of vitamin C intake.

Steps in Making Blood Analysis.

1. Extracting the Dye.

   100 mg. of dye, weighed out on the chemical balance, are placed in a folded filter paper. Boiling hot water (approximately 80cc.) is then poured through this.

2. Preparing Metaphosphoric Acid Solutions.

   The solid chemical is weighed out on a trip balance and made into a 2.5 percent and a 5 percent solution.

3. Diluting the Stock Dye.

   After the flask of stock dye has cooled, it is filled up to the 100 cc. mark. Five cc. are transferred to a 50 cc. volumetric flask to make

a 1:10 dilution. This will be standardized.

4. Weighing Out Ascorbic Acid Standard.

About 60 mg. of ascorbic acid is accurately weighed out on a chemical balance.

5. Making Up and Diluting Ascorbic Acid Standard.

A 5-cc. microburette reading to 0.01 cc. is filled with dilute dye. In one test tube 2cc. of dilute ascorbic acid solution is placed. In the other (blank), 2.5% HPo_3. Titration of the ascorbic acid standard is carried out until the first paint pink color shows. The amount of dye which must be added to the HPo_3 blank to give the same shade of pink is subtracted from the reading.

7. Apparatus for Collecting Capillary Blood.

A sufficient quantity of lithium oxalate to cover the end of a toothpick is placed in the micro-blood bottle.

8. Collecting Blood.

Seven or eight drops are required.

9. Mixing with Anticoagulant.

The blood is immediately stirred rapidly with a toothpick.

10. Centrifuging out Cellular Elements.

Micro-blood bottles are placed in centrifuge cups.

11. Removing Plasma.

Exactly 0.1 cc. of plasma is pipetted into a centrifuge tube.

12. Deproteinizing Plasma.

To the 0.1 cc. of plasma are added 0.1 cc. of distilled water and 0.2 cc. of 5% HPo_3. After this has been mixed it is then centrifuged.

13. Further Dilution of Standardized Dye for Blood Titration.

The 1:10 stock dye, which has been previously standardized, is diluted with

an equal amount of distilled water. Five-cc. portions of each are sufficient. This "dye for Blood" is first mixed in a test tube and is then transferred to a beaker.

14. Filling Special Microburette.

By turning the screw, the mercury is forced to the tip, where a droplet is seen. This expels all air. The beak of the instrument is then submerged in the dye, and the latter caused to fill the burette by reversing the motion of the screw.

15. Measuring Out Deproteinized Plasma for Titration.

Exactly 0.2 cc. of deproteinized plasma is measured into each end depression of the white tile. In the center is placed 0.2 cc. of 2.5% HPO_3 to serve as a blank.

16. Titrating.

Dye is run into the depressions from the burette, while the other hand stirs the sample. The first faint pink color, observed in good daylight, is the end point. The amount of dye required to give the blank the same color is deducted from the reading on the blood samples.

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TYPICAL MENU

Breakfast

Oatmeal
Bacon Gravy
Biscuits Butter
Syrup
Coffee Milk

Dinner

Boiled Meat
Pinto Beans Potatoes*
Cornbread
Buttermilk

Supper

Chicken Soup
Bread
One pt. Sweet Milk

*Occasionally turnip greens or slaw are substituted for potatoes.

COMPILATION TABLE OF PLASMA LEVEL OF VITAMIN C PER 100 MILLILITERS OF BLOOD

Subjects	Dietary Intake Mg.	Initial Fasting Plasma Level	Dietary Intake	Test Dose Mg.	3 hr. Plasma Level	Fasting Plasma Level	Average Dietary Intake	Daily Supplement Mg.	Fasting Plasma Level 1st week 1st	1st week 2nd	2nd week 3rd	2nd week 4th
1.	36	0.07	24	150	0.12	0.24	21	75	0.30	0.06	0.78	
2.	14	0.07										
3.	40	0.07										
4.	40	0.14										
5.	9	0.07	17	75	0.89	0.48						
6.	38	0.28	65	300	0.42							
7.	11	0.28	10	75	0	0.48						
8.	14	0.07	32	150	0	0.30	24	75	0.18	0	0.72	
9.	30	0.28										
10.	30	0										
11.	29	0.14	57	75	0	0.48	25	75	0	0.12	0.66	
12.	34	0.07										
13.	65	0										
14.	34	0.21	30	300	0.24	0.41		75	0.06	0.12		
15.	19	0.28										
16.	28	0.21	35	150	0.12							
17.	27	0	17	75	0.18	0.24						
18.	24	0.07	30	300	0.24	0.41						
19.	2	0	6	150	0.48	0.12						
20.	33	0										
21.	20	0	20	0								
22.	36	0	52	75	0.06	0.30						
23.	17	0										
24.	33	0										
25.	20	0.14	22	150	0.24	0.12						
26.	22	0.14										