A STUDY OF THE RESPONSE OF TWO PATIENTS WITH PARKINSONISM TO DOSES OF ASCORBIC ACID AND THIAMINE

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By

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ABSTRACT

The nutritional status of two patients with Parkinson's disease at the Ingham County Hospital was assessed. Daily food records were obtained. Urinary excretions of ascorbic acid and thiamine, and blood serum ascorbic acid concentrations were determined by chemical analyses.

The three-month study was divided into four successive periods during which the subjects were given daily oral administrations of (a) a placebo, (b) 400 mg. of ascorbic acid only, (c) 400 mg. of ascorbic acid and 3 mg. of thiamine, (d) 3 mg. of thiamine only. Prior to period (a) the subjects had received 200 mg. of ascorbic acid per day for one month or longer.

Evaluation of the daily food records suggested that both subjects consumed a low-iron diet. Their mean daily intakes of iron were 31 to 35 percent below the 12 mg. per day recommended by the National Research Council. The diets of both subjects furnished adequate amounts of protein, calcium, vitamin A, and riboflavin, but on the average supplied less ascorbic acid and niacin than the amounts recommended by the National Research Council. For one subject the mean daily intake of thiamine was below 1 mg. except during the final month of study. The caloric intake of one subject increased at the rate of approximately one-hundred calories per month throughout the study, while the other subject had a relatively constant intake.

Blood serum ascorbic acid values were 2.10 and 1.86 mg. per 100 ml. for the two subjects during period (a). An increase in blood serum ascorbic acid occurred during the periods when 400 mg. ascorbic acid were given daily. Values were decreased to 0.83 and 0.85 mg. per 100 ml. six weeks after termination of the ascorbic acid supplement.

The percentage responses to a test dose of ascorbic acid were 15.0 and 15.9. These values are below the range of values observed for healthy women on self-selected diets.

The percentage responses to a test dose of thiamine were 12.5 and 21.9, which is within the range of values observed in healthy women.

There were no significant alterations in the subjects well-being or physical strength, or psychological status attributable to the therapy as assessed from the clinical examinations of a physician, a physical therapist, and a psychologist.

The data on these two subjects do not permit the establishment of definite conclusions, but they appear to justify additional investigation of the ascorbic acid nutrition of Parkinson's subjects.

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INTRODUCTION

A nutritional survey of certain patients at the Ingham County Hospital was conducted in 1953, by the Foods and Nutrition Department of Michigan State University. This study indicated that three patients with Parkinson's disease had serum total ascorbic acid concentrations from 0.15 to 0.22 mg. per 100 ml. (26). These values are considerably lower than the 0.73 to 1.21 mg. per 100 ml. serum, that was reported for healthy women who were receiving generous intakes of vitamin C (48).

Investigators (8) have reported that blood serum ascorbic acid values reflect previous dietary intake. The dietary histories of these three patients with Parkinson's disease suggested that their vitamin C intakes prior to admission to the hospital had been reasonably satisfactory; and since they had been hospitalized less than one month at the initiation of the study, the low serum ascorbic acid concentrations merited further investigation.

In the fall of 1954, the Foods and Nutrition Department undertook a study of two of the patients with Parkinson's disease at the Ingham County Hospital. The urine of the subjects was analyzed for excretion of ascorbic acid, thiamine, and riboflavin, before and after the administration of an oral test dose of each vitamin. The percentage excretion of the

test dose of ascorbic acid was 4.1 and 7.4, respectively (24). The values of similar tests observed for healthy women on self-selected diets ranged from 26 to 81 percent (5). After eleven days of an oral supplement of 400 mg. of ascorbic acid per day, the patients' blood serum ascorbic acid concentrations were 1.70 and 1.72 mg. per 100 ml. These values correspond favorably with those reported for healthy women receiving a generous intake of this vitamin (48).

The percentage excretion of the test dose of thiamine was 1.9 and 1.6. These values are low in comparison with those obtained in healthy women, namely 12 to 38 percent (6). The excretion of a test dose of riboflavin was calculated to be 4.0 and 26.3 percent, respectively. The values observed for healthy women on self-selected diets range from 26 to 38 percent (6). During the period of vitamin administration the patients reported that they were "feeling better".

The data that has been collected concerning the nutritional status of Parkinson's patients was of two weeks or less duration. Due to the observed feeling of well-being of the two patients a more carefully controlled study of longer duration seemed justified.

The purpose of the present study has been to determine the urinary excretions of ascorbic acid and thiamine and the

concentration of blood serum total ascorbic acid of two patients with Parkinson's disease preceding and following the administration of test doses of the vitamins. Clinical evaluations were made of the patients' general well-being.

REVIEW OF LITERATURE

Parkinson's Disease

Parkinson's disease is not a discrete entity. It has been described as a slow, progressive degenerative disease of the nervous system characterized by rigidity of the skeletal musculature, fixidity in posture and a tremor of regular, slow rhythm. It is not a disease in the sense of being due to damage of a unitary physiologic system and exhibiting a constant symptomatology. The disease is also known as paralysis agitans, shaking palsy, and Parkinsonism (16).

According to Christian (16) the classical clinical description of the disease was reported by James Parkinson in 1817, and the disease was first described as a pathological change at the base of the brain by Manchot in 1904.

The etiology of the disease is unknown. Usually the disease occurs at ages past forty years, but it has been reported in persons whose ages are in the twenties. A history of the disease in the same family is found in approximately 4 to 16 percent of the reported cases (16).

The disease causes cell degenerations in the basal ganglia. There is wide diversity of opinion concerning the precise location within the basal ganglia of the pathological process.

There are several reports in the literature that present a description of the progress of Parkinsonism (16, 55). The disease begins gradually, usually with tremor in one or the other hand. In the majority of cases, the disease sets in abruptly after fright or trauma. Other early signs of Parkinsonism are the loss of the normal swinging of an arm while walking, and much fatigue.

The characteristic features of the disease are the expressionless face, slow movement of the lips, the elevated eyebrows and general facial immobility. The tremor is regular in rhythm and appears in the small muscles of the hands and feet, the lips, the tongue and mandible. Weakness is greatest when the tremor is most developed.

Movements of a patient with Parkinson's disease are characterized by great deliberation. These patients rise from chairs slowly in a stooping attitude with the head projecting forward. Their steps are short and hurried. Usually it is easier for these patients to run upstairs than walk. Handwriting is tremulous and often micrographic.

Some patients with Parkinsonism complain of subjective sensations of heat which may be present on one side only and associated with an increase of surface temperature. In other instances patients complain of cold. The mental condition rarely shows any change until late in the disease.

There is no known cure for the disease. The treatment is symptomatic, and is directed toward relief of the tremor and rigidity. Death from Parkinsonism is usually due to intercurrent infection, such as pneumonia.

Investigators have presented the data of studies that have involved the use of vitamin therapy for the treatment of Parkinsonism. Baker (1) treated 15 patients suffering from paralysis agitans with pyridoxine hydrochloride, supplemented in most cases by oral administration of brewers' yeast. The patients received 50 to 100 mg. of the vitamin intravenously at daily intervals over a period of two to four weeks. The author stated that neither the age of the patient nor the duration of symptoms seemed to have an effect on the treatment. The nature of improvement varied from patient to patient. The indices of improvement were decreased fatigability, better sleeping, and increased appetite. There was "some" improvement noted in six subjects, and only in one subject was there any objective benefit in the form of observable decrease in tremor and rigidity.

It was Baker's opinion that the small number of patients did not allow any definite conclusions to be reported.

Zeligs (66) reported the use of vitamin B_6 in the treatment of Parkinsonism. Daily doses of 50 to 100 mg. repeated one to three times were administered intravenously

to 15 patients. Several days later there was no change in the degree of muscular rigidity or tremor. On the basis of the data, Zeligs concluded that vitamin B_6 had no value for the treatment of Parkinsonism.

Doshay (21) pointed out that loss of weight is especially likely to occur among patients who are tense, excitable, and tremulous. He reported that a number of Parkinsonism patients have responded favorably to vitamin B_{12} injections of one mg. twice a week supplemented by thiamine hydrochloride given orally or parenterally.

Methods of Assessing Nutritional Status

Several techniques are available for use in the assessment of the nutritive intakes for individuals or groups. The main objective of a study of dietary habits, whether sociological, physiological, or economic, usually determines the techniques involved (38, 35, 61).

Stiebeling (61) has outlined the methods commonly used in this country to determine food intake. They are as follows: (a) food habit inquiry, (b) quantity of food commodity available for family consumption, (c) family food records with inventories, (d) family food list, and (e) nutrient intake of an individual.

Since this study is concerned with the dietary evaluation of two subjects, the emphasis in this section of the review of literature has been placed on method (e), the nutrient intake of an individual.

Various techniques have been used to estimate the nutrient intake of an individual. Some investigators have determined the dietary intake from recall records, in which the accuracy of the data has depended on the memory of the subject. Other investigators have instructed the subjects to record the amount of food eaten in household units, or weights in grams. The nutritive value of the food thus recorded has been calculated from tables of food values. A third method of determining nutritive intake consists of the chemical analysis of a representative sample of the food eaten.

Burke (9) has stated that although the most accurate method of studying food intake was the controlled balance study; the food intake as recorded by a trained interviewer using the diet history, will result in a satisfactory method of diet analysis. The author (9) pointed out that the qualifications of the interviewer were most important for obtaining valid data by use of the research dietary history. The interviewer should be a "person with an alert and inquiring mind, accuracy for detail, keen powers of observation, and sound basic training in the allied sciences and nutrition".

Burke and Stuart (10) suggested that the interviewer record the subject's usual pattern of food eaten in household measurements and list the food likes and dislikes. The history should then be followed by a three-day food record. Combined records are used to estimate a "typical" day's dietary intake. The diets are evaluated by a rating scale containing a range of values wide enough to take care of most inaccuracies in the diet history.

In 1954, Van Den Berg and Mayer (64) reported a study which compared the dietary intake as recorded from the one-day food record and the research dietary history. The subjects were thirty-five obese pregnant women from the Research Obesity Clinic of the Boston Lying In Hospital. The research dietary history gave caloric intakes greater than those given by one-day record, the difference being about 600 calories. Differences in estimates appeared to be due mainly to inaccurate description of composition and size of dishes on the one-day record, and to omission from the one-day record of foods commonly consumed on the other days.

Several studies reported in the literature have compared the data of a food record obtained by recall to similar data obtained by the weighed food record. In 1950, Ohlson and others (51) reported that different kinds of information were

obtained when more than one technique was applied in a given situation. The estimated mean intake of calories, protein, and calcium of 13 subjects as recorded by recall and weighed diets was presented. The data revealed that the apparent mean intake of all nutrients was greater when measured by recall than from weighed diets.

Apparent reasons for the increase of nutritive value as recorded by recall were: (a) there was less eating between meals during periods on the weighed diets than during periods of recall, (b) the size of the portion selected by many of the aging women appeared smaller than that of the younger women whose food habits formed the basis for the calculations of the Leichsenring and Donnelson food composition table, and (c) in the first weighed diet period the fear of social rejection if mistakes were made resulted in emotional tension which could have reduced the amount of food eaten.

Young and others (65) have reported a comparison of calculated nutritive value of measured food intake against the subject's estimation of intake from the data obtained in the Northeastern Regional Research Nutritional Status Project NE-4. The authors have pointed out that interpretations of the data were difficult due to the fact that it was impossible to follow a consistent or identical routine in collecting data. The subjects were at one of six experimental stations and varied in age and environment.

At the Maine Experiment Station, 25 eighth and ninth grade children were observed at school lunch on three consecutive days. They were aware that they were being observed and would be asked to recall what they had eaten.

In New Jersey the noon meal of 56 male industrial workers was the basis of comparison of measured and recorded dietary intake. The meal was served in the plant cafeteria. The authors stated that there was no means of checking plate waste therefore the assumption was made that all food served was eaten. Comparisons were made only on the basis of measured food items and did not include items such as bread and butter and cups of beverage. The subjects were unaware that they were being observed.

At the New York Experiment Station 16 children twelve to fourteen years of age were observed for one school lunch. The amount of food served was estimated by three advisors from the home economics department. The child's estimation was obtained by questioning and recall directly after the meal. The children were not aware that they had been observed.

At the Rhode Island Station, 14 college girls were studied for one day during which they recorded their food intake at the college cafeteria. Measured quantities of food were served and food not eaten was recorded. The subjects were unaware that they were being observed.

In West Virginia, 21 college students in the Infirmary were observed for one day. The food served was measured and the unused portions were subtracted. The students recorded their food intake. They were not aware that their eating habits were being observed. The nutritive value of the food was computed by Babcock's simplified method of dietary calculation.

Considerable variation was found between the nutritive values calculated from the estimated and from the measured food intakes. In only one state, Maine, did the estimation of half of the individuals yield calculated nutritive values within ten percent of those of the measured values. Approximately two-thirds of the subjects estimated their food intake in such a manner that the calculated nutritive value was within twenty percent of that of the measured intake. It was noted that the best estimations were made by subjects who were aware they were being observed. The authors (65) pointed out that "for only one group, Rhode Island college students, were there pronounced under-estimations; for practically every nutrient, the error of by far the greater majority of subjects was an overestimation".

The greatest ranges of deviation of the nutritive value from the measured intakes were found in the New York school children's recalls and in the recorded estimations of the

New Jersey workers and the West Virginia college students. There was no nutrient that could be singled out for all states as being estimated most poorly.

From these surveys the authors (65) concluded that in studying the nutrient intake of an individual, the subject's ability to estimate the food portions could be a large source of error. They recommended that in the absence of a weighed record, measurement of intake in household units would be the next most desirable procedure; and also "that it is wise to check subjects' estimations periodically and to let them know they are being checked".

Trulson (63) reported a study comparing dietary data collected by the seven-day record, by dietary interview of usual food practices, and three or more 24-hour recall diet forms. No comparison was made with weighed diets. Data was gathered from 47 subjects attending two Chicago clinics. The differences in the mean values of the foods and nutrients obtained by the three methods were not consistent. The highest mean value for milk intake was obtained by the interview method; the average of three or more 24-hour recalls provided the lowest mean value. The highest mean value for intake of foods high in carotene was the 7-day record method; the interview method provided the lowest mean value. The author stated that "it must be accepted that it is difficult, if not

impossible, to determine which method gives correct food intake". From the observations in the study, it was the author's opinion that the long-range interview of usual food practices was the method of choice, particularly in clinical studies.

In 1942, Huenemann and Turner (34) reported a study comparing data obtained by weighing and by interview method. Data was collected from 25 University of Chicago Clinic patients whose ages ranged from 6 to 16 years. Each patient was questioned regarding his usual food intake, his previous day's intake and the frequency with which specific foods were eaten. In addition, the family food purchases and distribution of food among the members of the family were determined. Three or four periods, each of 10 to 14 days duration, of weighed food intake were obtained.

When estimations of food intake obtained from the dietary history were compared with weighed dietary records, the authors found that no dietary history agreed with the weighed dietary record within twenty percent for all the nutrients. Approximately one-half of the histories differed from the weighed records in five or six dietary constituents by a deviation of twenty percent. On the basis of this study the author stated that data obtained by interview method should be checked by a quantitative record of food intake.

Several studies have been reported concerning the desirable length of the dietary study. In 1955, Trulson (62) reported the finding of dietary studies based on records gathered from one day, three days, and seven days. Information was obtained from a random sample composed of 132 Chicago school children whose ages ranged from 10 to 12 years. The results of the study revealed that lengthening the experimental period from one to three to seven days, reduced the standard deviation respectively. She stated that the length of the study should be determined by the nutrient or food to be investigated. A nutrient, such as protein, required shorter periods and smaller samples.

Chappell (15) stated that a short term survey should be interpreted with caution. His statement was based on data from a study of a 34-year-old woman observed for 70 weeks, and a 66-year-old man observed for 13 weeks. The weekly intake of calories and nutrients showed considerable variation differing with the dietary component and with the subjects. The woman's estimated average daily intake based on one week sampled at random within the year had a standard error of 8 to 15 percent for most of the dietary components but a much larger percentage of error for some of the vitamins.

The author stated that there was no reason to think the woman's dietary habits were particularly variable. Therefore, this suggested that the results of a short-term survey should be interpreted with caution.

A comparison of the food intake of college women for weekdays and for Saturday and Sunday was reported by Leverton and Marsh (39). The data of the study consisted of 24 balance studies made of college women, 17 to 24 years of age. The subjects were engaged in regular college work and light housekeeping.

Results indicated a definite and significant variation of food intakes for weekdays and for week ends when college women were living on a self-chosen diet. The least difference occurred between the period of five weekdays and the calendar week. The author concluded that studies of food intake of subjects on self-chosen diets would yield the most representative data when conducted for a period of at least one calendar week or in units of one week.

Church and others (17) have studied the ability of different interviewers to obtain comparable dietary survey data. The comparison of interviewers was made from material gathered by three state experiment stations participating in the Northeastern Regional Project NE-4. Data was collected in Maine by three interviewers, working as a team in each of

two schools. Two interviewers, in each of two industrial plants, collected the data obtained in New Jersey. In New York, the dietary histories were taken by two interviewers in one school.

The background of all the interviewers was similar. All were nutritionists who had had some training in dietary interviewing before collecting data for the project. The same set of instructions was given to all interviewers. From each subject, a diet history was taken and then verified by crosschecking from a list of food groups. The comparison was made between interviewers at the same station. The author stated that with the available data, the difference that may occur between interviewers at different stations could not be tested statistically.

The differences that occurred among interviewers rarely exceeded ten percent of the allowance for each nutrient, the average being 5.4 percent. It appeared that these differences were not likely to exceed sampling variations inherent in dietary survey methods, therefore, it was concluded that interviewers with similar background and training for cooperative research could obtain comparable data.

After food records have been obtained, their nutrient content is assessed. Recommended dietary standards have been proposed by the (a) National Research Council, United States of America, 1953, (23); (b) the Canadian Council of Nutrition, 1950, (11); and (c) the British Medical Association, 1950, (7). These three standards vary in philosophy and objective, therefore in specific recommendations.

The recommended dietary standards of the National Research Council (23) were designed

> "for the maintenance of good nutrition of healthy persons in the United States under present conditions. The recommendations are not requirements, since they represent not merely minimal needs of the average person but nutrient levels selected to cover individual variations in a substantial majority of the population".

The recommended dietary allowances were formulated by the Food and Nutrition Board of the National Research Council. The allowances were based on data published in the literature that related to the requirements for the various dietary essentials, and the combined judgment of a large number of nutrition authorities, particularly those actively engaged in research.

The dietary standards proposed by the British Medical Association (7) differ from the National Research Council Recommended Allowances. The British standard provides "values which apply to the average person rather than values which will

cover essentially all individuals, recognizing, however, that some members in every group may need more than the average".

The Canadian standards differ considerably from the American and British recommendation. The Canadian standard represents a "nutritional floor" beneath which maintenance of the health of the people cannot be assumed (11, 12, 13).

A possible source of error of the evaluation of dietary intake results from the fact that food composition tables differ. Hunscher and Macy (35) compared the analyzed value of vitamin A with those estimated from three standard tables. (Table I)

Differences between values obtained by analyses of a dietary intake and those estimated from standard tables result from several factors: (a) the numerous methods of chemical analyses of foods, (27, 40, 62), (b) losses of various nutrients during storage, preparation, and cooking, (14, 43) and (c) the different types of soil, mode of cultivation, amount of water available, geographic origin, and marketing conditions involved in food production (3, 45, 60).

Clear concepts are also lacking with regard to the methods used to calculate the amount of energy derived by the body from a given amount of food eaten.

TABLE I

COMPARISON OF THE ANALYZED VALUE OF VITAMIN A INTAKE WITH THOSE ESTIMATED FROM THREE STANDARD TABLES

Method	Intake of vitamin A per day
Analyzed	341 I.U.
Estimated	
Table 1	1238 I.U.
Table 2	852 I.U.
Table 3	1156 I.U.

The majority of studies have calculated energy values in which either the Rubner or Atwater factors were used. In general, all the studies done in the United States since 1900, particularly those using the Rose or Sherman tables are based on the Atwater factors.

Maynard (44) has reviewed the Atwater system of calculating the calorie value of diets. He pointed out that the factor of 4-9-4 was meant to apply to diets in which kinds and proportions of food were similar to the "average" mixed diet. The Atwater figures should not be applied to diets markedly different in make-up. "The figures 4-9-4 were never intended to be applied without modification to all diets" (44).

Throughout the study records of the food consumed by the patients were obtained in the following manner: a food record form listing the amount of a serving eaten was filled out daily by a member of the nursing staff of the hospital. This form was kept at the patient's bedside. This method reduced extra work for the nursing staff. Both patients were served their meals on trays in the same room.

Data gathered from other diet surveys indicated that this method is adequate for recording information required in this study.

The Leichsenring and Wilson (37) food composition table was used to estimate the nutritive value of the dietary intake of the two subjects. The reliability of this method had been demonstrated previously in this and other laboratories.

Leichsenring and Wilson evaluated the accuracy of the short methods table by the use of statistical tests. The observed differences between the short method and long method are presented in Table II. The data were based on 21 records gathered over a period of three days. From the use of statistical tests the observed differences were shown to be due to errors in random sampling and not to a real difference between the two methods.

Ohlson and others (51) in reporting the nutrition and dietary habits of 13 women subjects, 51 to 77 years of age, stated that the subjects' diets as computed by the short method of Donelson and Leichsenring "accurately reflected the results of laboratory analyses for nitrogen, calcium, and phosphorus except in cases of limited food intakes or where predominance of bread, processed meats or intricate cookery mixtures were found".

Ascorbic Acid Analysis

Early ascorbic acid research utilized the biological technique that was dependent on the prevention of scurvy in

TABLE II

COMPARISON OF THE MEAN VALUES OBTAINED WITH THE LONG AND SHORT METHODS OF DIETARY ANALYSIS*

Method	Calories	Protein (gm.)	Vitamin C (mg.)	Thiamine (mg.)
Long	1984	65.8	75.9	1.13
Short	2004	65.1	74.4	1.12
Difference	20	0.7	1.5	0.01

*Leichsenring and Wilson (37)

guinea pigs (59). This method was followed by a similar growth test (29). In 1947, Crampton (20) proposed a bioassay for ascorbic acid that involved the growth of the odontoblast cells of the incisor teeth of young guinea pigs. The applicability of this method is limited if the potency of the test material is too low for it to be measured accurately by feeding trials, or if the sample is poorly absorbed by the experimental animal.

In recent years biological methods of assay for ascorbic acid have been replaced largely by chemical methods (28, 49, 58). The technique most commonly used for the chemical determination of the vitamin consists of the measurement of the reducing capacity of ascorbic acid with 2, 6-dichlorophenolindophenol, carried out in an acid medium (4, 36, 47). A second technique involves the use of the osazone reaction, the estimation of total ascorbic acid using 2, 4-dinitrophenylhydrazine (42, 57). Micromethods of the latter have been adapted by Lowry and others (42), thus subjects have not needed to experience the trauma of venous punctures for blood ascorbic acid determinations.

Other methods include the photosensitized reaction with methylene blue, and the reaction with iodine which has been mainly of value in testing pure ascorbic acid or pharmaceutical preparations free from interfering substances (58).

According to Gyorgy (27) early reports of the indophenol method appeared in 1927, from Tillman and his group. They published the results of the estimation of oxidation-reduction potential and its application to food chemistry. This group devised the method of distinguishing natural from imitation fruit juices by observation of the strong reducing power of fresh lemon and other fruit juices against 2, 6-dichlorophenolindophenol.

Gyorgy (27) stated that Tillman showed that this reduction of the dye to the leuco form was a measure of the antiscorbutic activity of the lemon juice. From the investigations of other workers there resulted numerous modifications of Tillman's visual procedure. Harris and Ray (30) developed a specific quantitative test for the determination of ascorbic acid. Their method included the use of a preliminary extraction process with trichloroacetic acid (later replaced by metaphosphoric acid) and the subsequent titration of the ascorbic acid solution in a relatively strong acid solution. The titrant was the indophenol dye solution. At this time it became apparent that substances other than ascorbic acid reduced the dye to its leuco form. It was shown that the reaction of ascorbic acid was almost instantaneous while the reaction of the interfering substances proceeded at a slower rate.

Evelyn and others (22) believed that non-ascorbic acid reducing substances could account for more than ninety percent of the total indophenol reducing capacity of normal urine. They presented a method of differentiating between ascorbic acid and other reducing substances which was based on the differences in the rate at which the dye was decolorized. "It has been shown that the reaction of ascorbic acid is almost instantaneous, while that of other known interfering substances proceeds at a slower rate" (22). On the basis of their experience, they suggested a method in which an excess of the dye was mixed with a measured aliquot of urine, and the amount of dye decolorized at five second intervals was measured in a photoelectric colorimeter until the reaction was complete. These results were used to plot a reaction velocity curve from which the amount of decolorization due to ascorbic acid was determined by extrapolation to zero time. The authors stated that the method was empirical and could only be expected to give approximate results; however, they believed there were far less errors in this method than those encountered from the use of visual titration.

King (36) pointed out two crucial points to consider when using the indophenol method: (a) the avoidance of oxidative changes previous to the titration, and (b) awareness of circumstances under which other reducing substances will or

will not interfere with the color reaction. The indophenol dye is reduced by thiosulfate, cysteine, and other substances containing sulfhydryl groups.

Mindlin and Butler (47) have stated that in using the indophenol method the pH of the metaphosphoric acid extract must not be low enough to cause significant fading of the dye, and yet not so high as to result in immediate reduction of the dye by reducing substances other than ascorbic acid that are present in the extract.

Roe and Kuether (57) developed the method utilizing the osazone reaction which is based on a direct colorimetric determination of dehydroascorbic acid. The oxidized form of the vitamin in metaphosphoric acid extract is coupled with 2, 4-dinitrophenylhydrazine, and the resulting derivative is treated with sulfuric acid to produce the red-colored osazone which is measured photometrically. For the determination of total ascorbic acid the reduced ascorbic acid is first oxidized to dehydroascorbic acid.

Lowry and others (42) adapted the dinitrophenylhydrazine method for the determination of ascorbic acid in a small amount of serum.

Objections to the osazone method were reported by Pijoan and Gerjovich (53) who found that this method sometimes gave unreliable results for ascorbic acid content of certain biological

preparations if loss in lactone structure of dehydroascorbic acid had taken place. Penney and Zilva (52) showed that a 2,3-diketo-l-gulonic acid compound could be made from dehydroascorbic acid by mutarotation and loss of lactone structure. The d-glucoascorbic acid and d-araboascorbic acid could not be distinguished from l-ascorbic acid by the 2,-4-dinitrophenylhydrazine method.

Roe and Kuether (57) pointed out that oxidizing agents such as Fe^{+++} ions and H_2O_2 could produce interfering color substances with the 2,4-dinitrophenylhydrazine.

In 1951, Hewston and coworkers (33) compared three methods of determining ascorbic acid: the 2,6-dichlorophenolindophenol, 2,4-dinitrophenylhydrazine, and the bioassay method involving growth of the odontoblast cells of the incisor teeth of young guinea pigs. The authors stated that when extraneous reducing substances were present neither chemical procedure could be relied upon to give an accurate measure of vitamin C.

Total ascorbic acid in the blood serum was estimated according to the method of Bessey and Lowry as listed in the Northeast Regional Publication Number Five (19) except that the standard was prepared daily. This procedure is one of the most acceptable ones, since the amount of serum necessary for the determination is obtained from a finger prick blood

sample. Other advantages of this method were that laboratory facilities were readily available for the determination, and the data obtained in this study could be compared readily with data from similar studies on normal, healthy women.

Thiamine Analysis

Methods most often used to estimate thiamine are (a) chemical procedures involving either the oxidation of thiamine to thiochrome which can be measured by its fluorescence, or a colorimetric procedure based upon a coupling reaction with a diazotized amine, (b) the yeast fermentation procedure, (c) microbiological assays, and (d) biological assays using rats, chicks, or pigeons as test animals. Many modifications of these various procedures have been published.

Two of the early reports using thiochrome method appeared in 1935. Barger and coworkers (2) found that thiamine on oxidation with ferricyanide in alkaline solution was transformed into thiochrome. Robinson (56) reported that Jansen converted thiamine into thiochrome, extracted the thiochrome with isobutanol, and measured the fluorescence of the extract in a fluorometer calibrated against standard solutions of quinine.

Many biological fluids, such as urine, contain interfering substances that prevent accurate estimations of thiamine. Hennessy and Cerecedo (32) reported that interfering substances could be eliminated by adsorption of the thimaine on zeolite

and subsequent eluting with hot potassium chloride solution.

Mickelsen and others (46) pointed out that there is little information concerning the interfering fluorescent substances in urine, and that there may be other substances present which contribute to the total fluorescence and therefore, are read as thiochrome. Mickelsen and co-workers also made a statistical evaluation of the thiochrome method. They found that in 33 pairs of duplicate samples the standard deviation of the thiamine content was 4.6 percent of the mean.

Hennessy (31) reported that the oxidation of thiamine in alkaline ferricyanide solution did not result in the production of an equivalent amount of thiochrome. The amount of conversion was not reported. He suggested the incomplete conversion was due either to establishment of an equilibrium in the oxidation process or to a side reaction.

Conner and Straub (18) suggested the isobutyl alcohol be distilled between 106 and 107°C. which prevents any fluorescent material from being carried over into the distillate. A glass apparatus should be used.

In the present study the thiamine content of the urine of two Parkinson's patients was determined by the thiochrome technique of Conner and Straub (18). This method was chosen since it is one of the most accurate methods. Other advantages of using the thiochrome technique were that laboratory

facilities were readily available, and the previous data collected concerning the thiamine status of these patients had been estimated by the thiochrome technique.

EXPERIMENTAL PROCEDURE

Experimental Plan

Since this study was undertaken to determine the possible beneficial effects of ascorbic acid and/or thiamine, the following experimental plan was designed. The three-month study (Table III) was divided into four successive periods during which the subjects were given daily oral administrations of (a) a placebo, (b) 400 mg. of ascorbic acid only, (c) 400 mg. of ascorbic acid and 3 mg. of thiamine, (d) 3 mg. of thiamine only. Prior to Period (a), the subjects had received 200 mg. of ascorbic acid per day for one month or longer.

During Period a, a sugar placebo was given daily. Twenty-four-hour urine specimens were collected on three successive days per week for Period a and analyzed for the vitamin C content. The urine was collected in brown bottles which contained metaphosphoric acid, sulfuric acid, and 8-hydroxyquinoline. The serum ascorbic acid concentration of each subject was determined from fasting blood samples collected on the last day of Period a.

On the first day of Period b, the 24-hour test dose response of ascorbic acid was calculated. The formula used to calculate percentage response of a test dose of the vitamin was as follows:

$$\% \text{ test dose} = \frac{\left[\text{Excretion after test dose (24 hr.)}\right] - \left[\text{Basal excretion (Avg. for 24 hr.)}\right]}{\text{Test dose}} \times 100$$

The administration of 400 mg. of this vitamin was continued throughout the 28-day period in an attempt to saturate the patients. Analyses of the urinary excretion of the vitamin were made from three 24-hour urine collections per week. The serum ascorbic acid level was determined from a fasting blood sample collected at two-week intervals throughout the study. In the latter part of Period b, the basal urinary thiamine excretion was measured from 24-hour urine specimens collected during three successive days per week for a two-week period.

On the first day of Period c, the percentage response to a 3-mg. test dose of thiamine was determined. Throughout this period, 400 mg. of ascorbic acid and 3 mg. of thiamine were given daily. The urinary concentrations of thiamine and ascorbic acid were determined from 24-hour collections. A maximum of three collections were made each week.

During Period d, the administration of ascorbic acid was discontinued, but the daily dose of 3 mg. of thiamine continued. Urinary concentrations of thiamine were determined from 24-hour collections twice per week. Serum ascorbic acid was measured periodically.

TABLE III

METHOD OF ADMINISTRATION OF ESTIMATION OF ASCORBIC ACID AND THIAMINE

Period	Length of time (days)	Vitamin Supplement
a	18	None
b	28	400 mg. ascorbic acid per day
c	30	400 mg. ascorbic acid, and 3 mg. thiamine per day
d	28	3 mg. thiamine per day

Clinical evaluations by a physician, occupational therapist, and psychologist were scheduled for each period.

Analytical Procedures

The 2,6-dichlorophenolindophenol method was used to determine the concentration of ascorbic acid in the urine. The method of Evelyn and others (22) was followed with some modification. A calibration curve was used to calculate the ascorbic acid content of the urine. The curve was prepared from eight standard ascorbic acid solutions; the concentrations of which ranged from 5 to 40 mcg. per 5 ml. The concentration of urine was adjusted with one precent metaphosphoric acid solution to the degree that permitted the galvanometer reading of a 5 ml. sample to be within the linear portion of the calibration curve. Frequent calibration curves using standard ascorbic acid solutions were prepared to assure stability of the indicator solution. To remove any error which is due to a possible decomposition of the dye, fresh solutions of dye were prepared daily.

The dye solution was standardized as follows: Five ml. of one percent metaphosphoric acid were added from a blow pipette to a colorimeter tube containing 5 ml. of dye solution. Galvanometer readings were taken at five second intervals. The dye solution was diluted so that the reading on the galvanometer scale was approximately fifty.

Five ml. of diluted urine were added to a colorimeter tube containing 5 ml. of standard dye solution, mixed, and quickly placed in the cuvette holder of the instrument. Galvanometer readings were taken at five-second intervals. Three urine dilutions were made from each 24-hour urine collection. Duplicate measurements of each urine solution were made.

The galvanometer values of the urine samples were plotted against time on linear graph paper and extrapolated to zero time. The micrograms of ascorbic acid in a 5-ml. sample were read directly from the calibration curve.

Fasting blood samples of each subject were collected on September 15, and every two weeks thereafter until December 19. The 2,4-dinitrophenylhydrazine procedure based on that of Lowry and others (41) was used for the determination of total ascorbic acid in the serum samples. The method as described in the Northeast Regional Publication Number 5 (19) was modified in that the standards were prepared fresh daily rather than frozen simultaneously with the serum samples. The determination is dependent upon the separation of dehydro-ascorbic acid as an osazone derivative of 2,4-dinitrophenlhydrazine. When the osazone is treated with sulfuric acid a color is produced, the transmittance of which shows good agreement with Beer's law.

The blood samples were obtained in the following manner: the subject's hand was placed in a basin of warm water for approximately five minutes in order to stimulate circulation of the blood and then a finger was pricked with the point of a surgical blade. A few drops of blood were collected in a small glass tube and immediately centrifuged. Using a Levy Lang pipette three 10-mm^3 samples of serum were placed into serological tubes, and 40-mm^3 of five percent trichloroacetic acid were added. The samples were mixed, capped, and placed in ice, and after approximately thirty minutes placed in storage in the deep freeze.

The thiamine content of the urine was determined by the thiochrome technique. The procedure followed was that reported by Mickelsen and others (46). The urine was passed through a column of Decalso.* Modifications of the oxidation step were made according to recommendations reported by Conner and Straub (18).

The oxidation of thiamine to thiochrome was accomplished as follows: (a) 3 ml. of 15 percent sodium hydroxide and 0.1 ml. of 1 percent potassium ferricyanide were placed in an oxidizing chamber, (b) 5 ml. of eluate were transferred to the oxidizing chamber and 15 ml. of isobutyl alcohol were

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*The Permutit Co., 330 West 42nd Street, N. Y.

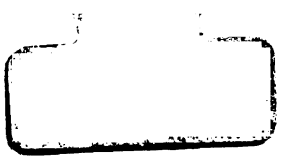
added, (c) the solution was shaken for 1.5 minutes, and then centrifuged for 1.5 minutes, (d) the aqueous layer was removed with a pipette, and two grams of anhydrous sodium sulfate were added, and the solution was centrifuged for one minute. The isobutyl alcohol solution which contained the thiochrome was transferred to a cuvette, and the amount of light transmission read in the Coleman fluorophotometer. The instrument was standardized against a quinine sulfate solution.

Estimation of Food Intake

Throughout the study, records of food consumed by the two patients were obtained by a food record form (appendix) for each patient, which was filled out daily by a member of the nursing staff of the hospital. From these records, the nutrient value of the food consumed was calculated with the use of the short form of Leichsenring and Wilson (37). The average daily intake of nutrients in the food consumed during the first week of each month was considered to be representative of that for the entire month.

Administration of Vitamins

The vitamins were given orally. Gelatin capsules were filled with a weighed amount of cerelose-vitamin mixture so that the capsules contained either 400 mg. of ascorbic acid, or 3 mg. of thiamine. To assure correct dosage, quantitative determinations were made from capsules which were selected by random sampling. The error in the weight of ascorbic acid was found to be ± 3.5 percent and of thiamine, ± 2.5 percent. Capsules were also filled with cerelose which were administered as a placebo during the first period of the study. Throughout the study the patients were not aware of the content of the capsules.



RESULTS AND CONCLUSIONS

Evaluation of the Foods Records

The nutritive value of the food intakes of the two patients was estimated according to the short form of Leichsenring and Wilson (37). These data are presented in Table IV. Both subjects knew that daily records were kept. This awareness may have influenced their intakes since they took considerable interest in the study, and as roommates, usually compared foods eaten at each meal.

The diets of the two subjects furnished the National Research Council recommended allowances of calcium, protein, vitamin A, and riboflavin. Both subjects consumed a low-iron diet. The mean daily intakes of iron were 8.5, 8.3, 8.0, and 8.4 mg. in September, October, November, and December, respectively, for subject H.L.; and 7.3, 7.4, 7.7, and 8.8 mg. respectively, for subject P.P. These mean daily intakes of iron were 31 and 35 percent below the National Research Council recommended daily allowance of 12 mg. for a sixty-year-old woman. In future work with Parkinson patients it would be desirable to include the determination of hemoglobin as this provides a means of estimating the adequacy of dietary iron.

TABLE IV

THE AVERAGE NUTRITIVE VALUE OF FOOD CONSUMED BY TWO SUBJECTS WITH PARKINSON'S DISEASE

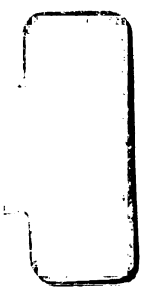
Subject H.L.

Nutritive Value of		Month Sept.	Oct.	Nov.	Dec.	N.R.C. Recommended Allowances
Protein	(gm)	63.5	63.5	68.2	64.8	55.0
Calcium	(gm)	1.12	1.10	1.11	1.13	0.80
Iron	(mg)	8.5	8.3	8.0	8.4	12.0
Vitamin A	(I.U.)	10,900	3,235	3,130	5,884	5,000
Thaimine	(mg)	1.00	1.07	1.02	1.04	1.00
Riboflavin	(mg)	2.20	1.80	1.86	1.96	1.40
Niacin	(mg)	9.32	9.24	9.40	9.55	10.00
Ascorbic Acid	(mg)	77	62	48	63	70
Calories		1863	1874	1856	1878	1800

Subject P.P.

Nutritive Value of		Month Sept.	Oct.	Nov.	Dec.	N.R.C. Recommended Allowances
Protein	(gm)	60.0	55.9	64.4	69.4	55.0
Calcium	(gm)	1.03	1.03	1.10	1.17	0.80
Iron	(mg)	7.3	7.4	7.7	8.8	12.0
Vitamin A	(I.U.)	10,147	4,159	3,530	4,383	5,000
Thiamine	(mg)	0.83	0.88	0.83	1.21	1.00
Riboflavin	(mg)	2.18	1.85	1.86	2.25	1.40
Niacin	(mg)	8.71	8.80	10.13	11.11	10.00
Ascorbic Acid	(mg)	80	53	57	68	70
Calories		1644	1716	1831	1964	1800

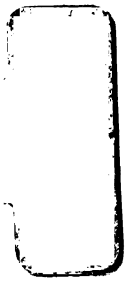
*The National Research Council Recommended Dietary Allowances of the above constituents for a 60-year-old woman.



The National Research Council has recommended an intake of 10 mg. of niacin per day for women 65 years or older. For subject H.L. the mean daily intakes of niacin were 9.32, 9.24, 9.40, and 9.55 mg. in September, October, November, and December, respectively; and for subject P.P. 8.71, 8.80, 10.13, and 11.11 mg., respectively.

The greatest day-by-day variation was observed in the intake of vitamin A. For subject H.L. the mean daily intake varied from 3, 130 I.U. during November, to 10,900 I.U. during September. Comparable values for subject P.P. ranged from 3,530 I.U. (November daily mean) to 10,147 I.U. (September daily mean). The mean intakes during the four months study were 5,787 I.U. and 5,555 I.U. for subjects H.L. and P.P., respectively. Similar findings of such variation have been reported in other nutritional surveys. It is generally accepted that the body has the capacity to store vitamin A; therefore, the average intake of this vitamin was judged to be satisfactory.

The mean daily intakes of thiamine for subject P.P. during September, October, November, and December, were 0.83, 0.88, 0.83, and 1.21 mg., respectively. With the exception of the final month of the study the daily intakes of thiamine were lower than the one mg. per day recommended by the National Research Council. The mean daily intakes of



thiamine for subject H.L. during the four months of the study were 1.00, 1.07, 1.02, and 1.04 mg., respectively.

Both subject's intakes of ascorbic acid were the greatest during the first month of the study when cantaloupe was available. In September the mean daily intake of this vitamin was 77 mg. for subject H.L., and 80 mg. for subject P.P. For both subjects the mean daily intakes of ascorbic acid the following three months of the study were below the 70 mg. recommended by the National Research Council. For subject H.L. the mean daily intakes of ascorbic acid were 62, 48, and 63 mg. in October, November, and December, respectively. The comparable values for subject P.P. were 53, 57, and 68 mg., respectively.

Subject H.L. had a relatively constant caloric intake throughout the four months of the study. Her mean daily caloric intakes were 1863, 1874, 1856, and 1878 calories during September, October, November, and December, respectively. No significant weight change was observed. Subject H.L. weighed 147 pounds in September, and 150 pounds in April.

The caloric intake of subject P.P. increased at the rate of approximately one-hundred calories per month throughout the study. Her mean daily caloric intakes were 1644, 1716, 1831, and 1964 during September, October, November, and December, respectively. Although no weight

records were obtained for this bedfast patient, it can be assumed that her caloric intake was in excess of her energy expenditure since the nurses reported that she was gradually becoming too heavy for nursing care. Ohlson and others (50) studied the dietary habits of aging women and reported that it was possible that the total food needs were lower after seventy. It appeared that this reduction occurred rather abruptly in response to decreased number of activities and interests. These observations suggest that further study is needed in order to fully understand the nutritional requirements of patients who must decrease activity, such as those confined to bed.

Serum Ascorbic Acid Concentrations

The serum ascorbic acid concentrations of the two patients were determined on the various regimens. These values along with similar data gathered in two previous studies in this laboratory (24, 26) are presented in Table V.

None of the serum ascorbic acid values recorded in the present study are below the range of 0.73 to 1.21 mg. per 100 ml. of serum observed in healthy women on self-selected diets (48). On September 15, 1955, the serum ascorbic acid concentrations were 2.10 mg. for subject H.L., and 1.86 mg. for subject P.P. A factor to be considered when interpreting

TABLE V

SERUM ASCORBIC ACID CONCENTRATIONS

Date	Daily Vitamin Supplement (mg.)		Serum Ascorbic Acid (Mg./100 ml. of serum)	
	Ascorbic Acid	Thiamine	Subject H.L.	P.P.
Spring of 1954[+]	none	none	0.15	0.22
Fall of 1954*	400	none	1.72	1.70
Fall of 1955				
September 15	**none	none	2.10	1.86
September 28	400	none	2.52	2.64
October 13	400	3	2.64	2.43
October 27	400	3	2.98	3.43
November 10	none	3	2.39	2.54
November 23	none	3	1.62	1.75
December 8	none	none	1.12	0.87
December 19	none	none	0.83	0.85

[+] Food and Nutrition Department (24).

* A daily oral supplement of 400 mg. of ascorbic acid had been given for the preceeding 11 days. Furnival (26).

** Prior to September 1, both subjects had received 200 mg. of ascorbic acid per day for a period of one month or more. From September 1 to September 15, no supplements were given.

these data is that both subjects had received 200 mg. of ascorbic acid per day during August. The 200 mg. dose was discontinued on September 1, thus there was a two-week period when no vitamin supplement was given. It is likely that the serum ascorbic concentration of the Parkinson's patients recorded on September 15, partly reflect the 200 mg. supplement administered during August.

During the period of the oral supplementation of ascorbic acid, the serum values for both subjects were above 2.4 mg. per 100 ml. of serum. The serum values for subject H.L. were 2.52, 2.64, 2.98, and 2.39 mg. on September 28, October 1, October 27, and November 10, respectively. For subject P.P. the serum values were 2.64, 2.43, 3.43 and 2.54, respectively.

After the discontinuation of the ascorbic acid supplementation on November 10, a downward trend in the serum values for both subjects was observed. The serum ascorbic acid concentrations for subject H.L. decreased from 2.39 mg. percent on November 10, down to 0.83, mg. percent on December 19. Similarly the values for subject P.P. declined from 2.54 mg. percent down to 0.85 mg. percent.

From October 1 to the 27, daily supplements of 3 mg. of thiamine and 400 mg. of ascorbic acid were administered to the Parkinson's subjects. The serum values recorded in this study reflected no apparent synergistic effect of these two vitamins.

In previous studies (24, 26) of these subjects the serum ascorbic acid concentrations were 1.72 mg. for subject H.L., and 1.70 mg. for subject P.P. during the fall of 1954; 0.15 mg. and 0.22 mg. respectively, during the spring of 1954.

As previously stated the serum ascorbic acid values recorded in the present study, during and immediately after the period of ascorbic acid supplementation, were above 2.4 mg. percent which is considerably above the values of 0.73 to 1.21 mg. percent reported for healthy subjects (48).

Lowry and others (42) reported that ascorbic acid plasma levels may rise to as much as 1.4 mg. percent on high intakes of the vitamin, and that, in general, the kidney excretes most of the larger doses, consequently the blood level does not rise farther. Pijoan and Lozner (54) stated that plasma ascorbic acid levels indicate essentially an overflow of a phase of positive balance, and that when this becomes high enough, urinary excretion of the vitamin takes place. Friedman and others (25) observed that plasma concentrations above 1.5 mg. percent resulted in an increase of urinary excretion of vitamin C. They found that below this plasma level urinary clearance of the vitamin was of small magnitude.

Responses to Test Doses of Vitamins

The percent responses to test dose of ascorbic acid and thiamine are presented in Table VI. Data from the fall of 1954 are also included (24).

TABLE VI

RESPONSES TO TEST DOSES OF VITAMINS

Vitamin	Date	Subject	Percent Response
Ascorbic Acid*	1954	P.P.	4.1
Ascorbic Acid	1955	P.P.	15.9
Ascorbic Acid*	1954	H.L.	7.4
Ascorbic Acid	1955	H.L.	15.0
Thiamine*	1954	P.P.	1.9
Thiamine	1955	P.P.	21.9
Thiamine*	1954	H.L.	1.6
Thiamine	1955	H.L.	12.5

*Furnival (26).

On September 15, 1955, the percent responses to test doses of ascorbic acid were 15.0 and 15.9 for subjects H.L. and P.P., respectively. These data are below the range of 26 to 81 percent response observed for healthy women on self-selected diets (5). Values from the fall of 1954 were 7.4 and 4.1 percent for subjects H.L. and P.P., respectively.

A low basal urinary excretion of the vitamin was observed at the time the test dose was given. The average 24-hour urinary excretion of a three-day period, week of September 12, 1955, for subject H.L. was 5.9 mg., and for subject P.P. 3.3 mg. Following the daily oral dose of 400 mg. of ascorbic acid, the urinary excretion of the vitamin increased. For subject P.P. the greatest excretion of ascorbic acid was observed during the week of October 25, with an average of 317 mg. per day for two 24-hour urine collections. For subject H.L. the maximum excretion occurred during the week of September 27, with an average of 217 mg. for three 24-hour urine collections.

The relatively high serum ascorbic acid values observed for these subjects along with the relatively low urinary excretion of ascorbic acid following a test dose might indicate a disturbed metabolism of ascorbic acid, particularly with relation to kidney function. However, there is a possibility that the total ascorbic acid values determined by the Roe and

Kuether method (57) may have included substances other than ascorbic acid which reacted with the 2,4-dinitrophenylhydrazine. Such substances may have arisen from drugs or medication and would not ordinarily appear in blood serum of healthy individuals. The fact that the rise and fall of the blood serum ascorbic acid values paralleled the period of supplementation, however, would indicate that the substance measured was ascorbic acid.

Further the fact that the test dose of ascorbic acid was of the same magnitude as the daily supplement may have accounted for the relatively small increase in urinary excretion of ascorbic acid after the test dose.

Certainly these findings are only indicative of the need for further exploration of the metabolism of ascorbic acid in patients with Parkinson's disease.

During the fall of 1955, the percent responses to the test doses of thiamine were within the 12 to 28 percent range observed in healthy women on self-selected diets (6). The values obtained were 12.5 and 21.9 percent for subjects H.L. and P.P., respectively. In the study (24) of the fall of 1954, the percent responses to a similar test dose were 1.6 for the subject H.L. and 1.9 for subject P.P. No explanation is apparent for the differences in response obtained in 1954 and

1955, however, a contributing factor may have been the levels of thiamine supplied by the daily diet. During the 1955 study, the diet of subject H.L. furnished over 1 mg. of thiamine per day, and that of subject P.P. averaged a little less than 1 mg. daily. Since thiamine intakes were not obtained in 1954, no conclusive statement can be made.

Clinical Evaluations

The physical well-being of the subjects was assessed from clinical examinations* of a physician, a physical therapist, and a psychologist. The physician saw both subjects on October 10, November 7, and December 18, 1955. He reported that there were no changes either in the neurological findings or blood pressure or general well-being of the subjects. During each visit a specimen of the signature of each subject was obtained. No significant differences were observed.

The physical therapist examined the subjects on August 29, September 6, October 2, and November 30, 1955, and reported no significant changes in the physical strength of either subject. Due to paralysis of the lower extremeties of subject P.P., testing was difficult.

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*The author is indebted to Dr. Mansel Dunn, Lansing, Michigan, for the general physical examinations; to Mr. Face, Ingham County Hospital, for the physical therapist's reports; and to Dr. Fink, Ingham County Hospital, for the psychologist's report.

The psychologist observed no psychological changes attributable to the therapy.

The data on these two subjects do not permit the establishment of definite conclusions, but they appear to justify additional investigation of the ascorbic acid nutrition of Parkinson's subjects.

SUMMARY

Literature pertinent to the etiology, symptoms, and treatment of Parkinson's disease was reviewed.

Methods of assessing nutritional status were described.

The nutritional status of two patients with Parkinson's disease at the Ingham County Hospital was assessed. Daily food records were obtained. Urinary excretions of ascorbic acid were determined by the 2,6-dichlorophenolindophenol method, and of thiamine by the thiochrome method. Serum ascorbic acid concentrations were estimated by the 2, 4-dinitrophenylhydrazine method.

The three-month study was divided into four successive periods during which the subjects were given daily oral administrations of (a) a placebo, (b) 400 mg. of ascorbic acid, (c) 400 mg. of ascorbic acid and 3 mg. of thiamine, and (d) 3 mg. of thiamine.

Evaluation of the daily food records suggested that both subjects consumed a low-iron diet. Their mean daily intakes of iron were 31 and 35 percent below the 12 mg. per day recommended by the National Research Council. The diets of both subjects furnished adequate amounts of protein, calcium, vitamin A, and riboflavin, but on the average supplied less ascorbic acid and niacin than the amounts recommended by the National Research Council. For one subject the mean daily

intake of thiamine was below one mg. except during the final month of the study. The caloric intake of one subject increased at the rate of approximately one-hundred calories per month throughout the study, while the other subject had a relatively constant intake.

Blood serum ascorbic acid values were 2.10 and 1.86 mg. per 100 ml. for the two subjects during period (a). An increase in blood serum ascorbic acid occurred during the periods when 400 mg. ascorbic acid were given daily. Values were decreased to 0.83 and 0.85 mg. per 100 ml. six weeks after termination of the ascorbic acid supplement.

The percentage responses to a test dose of ascorbic acid were 15.0 and 15.9. These values are below the range of values observed for healthy women on self-selected diets.

The percentage responses to a test dose of thiamine were 12.5 and 21.9. This is within the range of values observed in healthy women.

There were no significant alterations in the subjects well-being or physical strength, or psychological status attributable to the therapy as assessed from the clinical examinations of a physician, a physical therapist, and a psychologist.

The data on these two subjects do not permit the establishment of definite conclusions, but they appear to justify additional investigation of the ascorbic acid nutrition of Parkinson's subjects.

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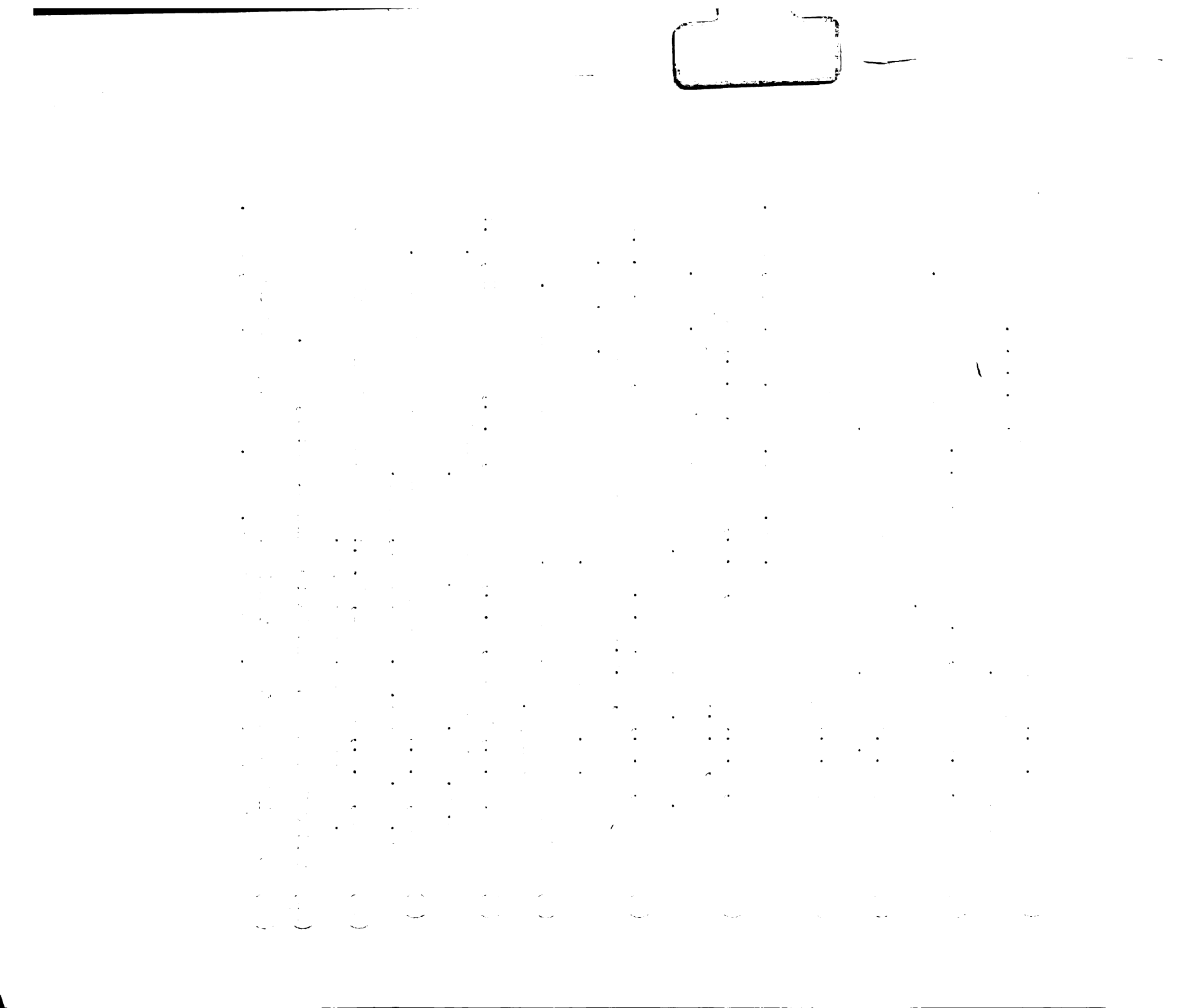

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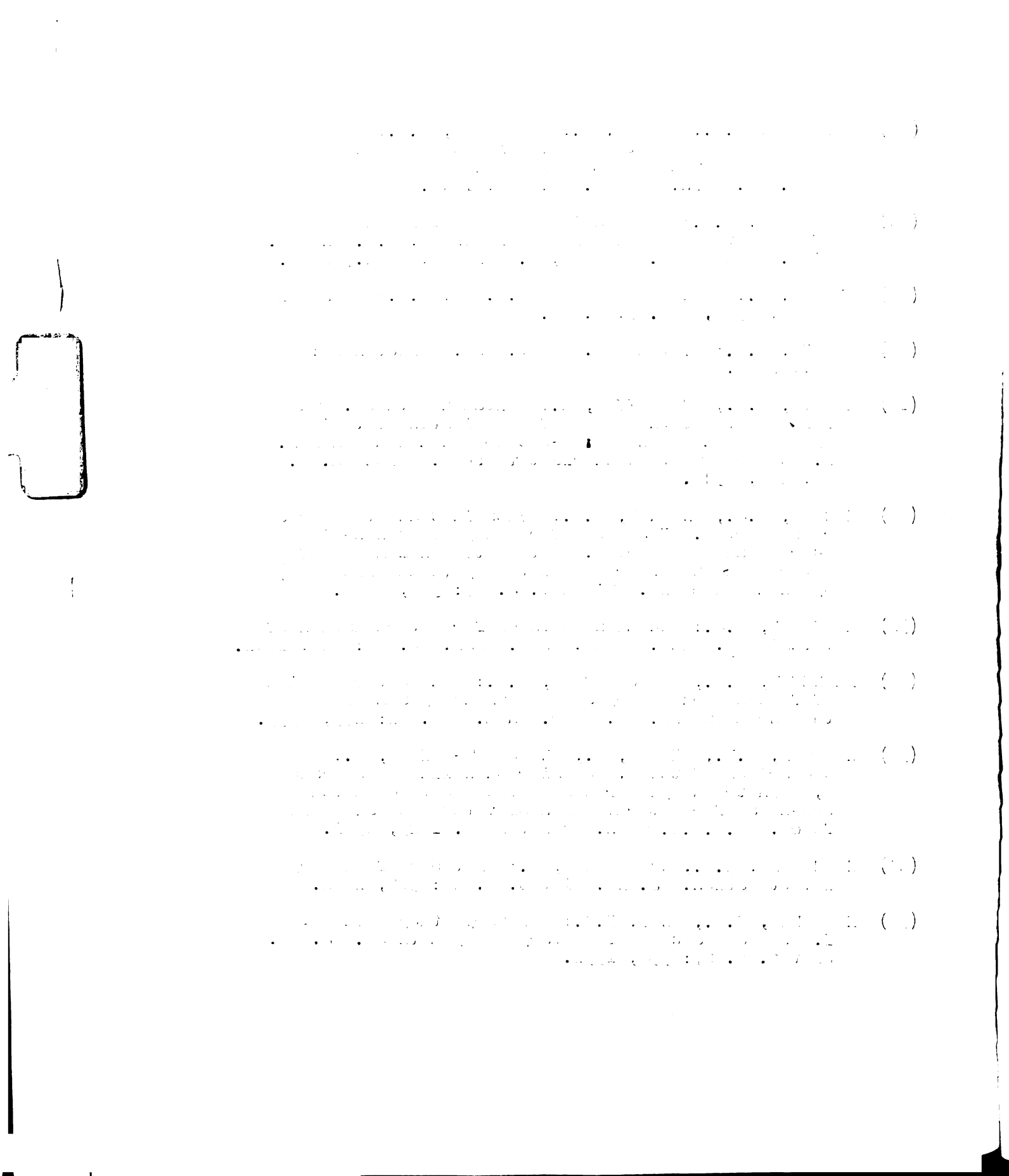

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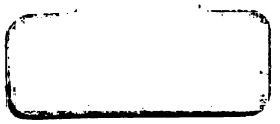
APPENDIX



Food Record Form RESEARCH

Name_____
Date_____
Day of Week_____

	Food Eaten	Amount Serving Eaten	Subjects' Comments
Breakfast			

What foods were on the tray that subject did not eat?
What did subject eat between breakfast and lunch?

Lunch			

What foods were on the tray that subject did not eat?
What food did subject eat between lunch and dinner?

Dinner			

What foods were on the tray that subject did not eat?
What did subject eat before going to bed?

Form recorded by _____(initials)

ABSTRACT

The nutritional status of two patients with Parkinson's disease at the Ingham County Hospital was assessed. Daily food records were obtained. Urinary excretions of ascorbic acid and thiamine, and blood serum ascorbic acid concentrations were determined by chemical analyses.

The three-month study was divided into four successive periods during which the subjects were given daily oral administrations of (a) a placebo, (b) 400 mg. of ascorbic acid only, (c) 400 mg. of ascorbic acid and 3 mg. of thiamine, (d) 3 mg. of thiamine only. Prior to period (a) the subjects had received 200 mg. of ascorbic acid per day for one month or longer.

Evaluation of the daily food records suggested that both subjects consumed a low-iron diet. Their mean daily intakes of iron were 31 to 35 percent below the 12 mg. per day recommended by the National Research Council. The diets of both subjects furnished adequate amounts of protein, calcium, vitamin A, and riboflavin, but on the average supplied less ascorbic acid and niacin than the amounts recommended by the National Research Council. For one subject the mean daily intake of thiamine was below 1 mg. except during the final month of the study. The caloric intake of one subject increased at the rate of approximately one-hundred calories per month throughout the study, while the other subject had a relatively constant intake.

Blood serum ascorbic acid values were 2.10 and 1.86 mg. per 100 ml. for the two subjects during period (a). An increase in blood serum ascorbic acid occurred during the periods when 400 mg. ascorbic acid were given daily. Values were decreased to 0.83 and 0.85 mg. per 100 ml. six weeks after termination of the ascorbic acid supplement.

The percentage responses to a test dose of ascorbic acid were 15.0 and 15.9. These values are below the range of values observed for healthy women on self-selected diets.

The percentage responses to a test dose of thiamine were 12.5 and 21.9, which is within the range of values observed in healthy women.

There were no significant alterations in the subjects well-being or physical strength, or psychological status attributable to the therapy as assessed from the clinical examinations of a physician, a physical therapist, and a psychologist.

The data on these two subjects do not permit the establishment of definite conclusions, but they appear to justify additional investigation of the ascorbic acid nutrition of Parkinson's subjects.