A QUANTITATIVE ESTIMATION OF THE EFFECT OF RUTIN ON THE BIOLOGICAL POTENCY OF VITAMIN C

by

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ABSTRACT

Considerable evidence has been advanced in the literature that rutin, and other vitamin P-active materials, act as antioxidants. The beneficial effect of some of these materials on vitamin C has been shown, but the results have been predominantly qualitative. This study, involving the administration of vitamin C to 128 guinea pigs, was designed to determine the extent to which rutin enhanced the apparent biological value of ascorbic acid. The odontoblast method of vitamin C bioassay was used.

Rutin increased the apparent biological value of ascorbic acid by approximately 50%, when the vitamin was supplied in sub-optimum amounts for normal tooth development, either in crystalline form or from a natural source. As the level of ascorbic acid intake, without rutin, approached optimum, the beneficial effect of added rutin was negligible. These results could be explained by the assumption that rutin prevented "in vivo" oxidative destruction of the administered ascorbic acid.

INTRODUCTION

Scurvy in humans was one of the first diseases to be traced to a deficient diet, and ascorbic acid was the first of the vitamins to be isolated, identified and synthesized. Thus, recognition of the necessity of a daily supply of vitamin C in the diet for certain species is not modern. Later research into the nature of the vitamin, however, has shown that ascorbic acid in a natural source may be readily oxidized to biologically inactive components under certain conditions, such as storage, cooking, etc. Consequently, further knowledge as to substances capable of preventing this oxidative destruction of vitamin C is of considerable importance.

With the identification of vitamin P-active materials in 1936, evidence of their relationship with vitamin C was advanced. At that time, vitamin P and ascorbic acid were thought to act synergistically in the prevention of scurvy, but more recent findings point to both having a distinct, though related, role in nutrition.

Furthermore, it now has been shown that when rutin, an established vitamin P-active substance, was administered with sub-minimum amounts of ascorbic acid, the life of scorbutic guinea pigs was prolonged. This discovery emphasized the already prevailing concept that rutin, and allied materials, acted physiologically as antioxidants. Therefore, the object of the work reported

herein was to further the present knowledge of the relationship of a vitamin P-active material (rutin) and vitamin C.

REVIEW OF LITERATURE

a) Isolation and Identification of Vitamin P-Active Materials.

The nature and importance of vitamin P-active materials have been reported extensively in the literature since the middle of the last decade. The early findings were varied and sometimes contradictory, and as a result, the concept of the function and mode of action of these materials was obscure. However, their role has become considerably clarified, and recent work has brought forth a theory as to the mode of action of the many substances possessing such activity.

That capillary walls become weakened under certain conditions has been known for some time. The decreased strength of the capillaries leads to hemorrhage, either through rupture of the walls, or because of their increased permeability. Prior to 1936, the belief existed that weakened capillaries were due wholly to a deficiency of vitamin C. In fact, capillary fragility was considered to be a part of the scurvy syndrome. However, such misconceptions were soon to be clarified.

In 1936, Szent-Gyorgyi isolated a substance from both lemons and fresh paprika peppers that would restore weakened

capillaries to normal. This effect was not noted when ascorbic acid alone was administered. Szent-Gyorgyi called his substance citrin, and the active principle thereof became known as vitamin P. In attempting to isolate this active principle, Bruckner and Szent-Gyorgyi (1936) stated that the extract was composed of the flavonones hesperidin and eriodictin. When the biological activity of these components was tested by Bentsath et al (1937), both were found to be active; while a related compound, a flavonol named quercitrin, was inactive.

In England, the work of Scarborough has been of considerable significance. In 1938, Scarborough and Steward found that the oral administration of hesperidin reduced the number of hemorrhages in patients with vitamin C deficiency. This effect was observed when petechial hemorrhages were induced by the application of negative pressure to the skin surface. Both Scarborough (1939) and Zacho (1939), writing independently, showed that capillary fragility was not controlled by the administration of ascorbic acid, but that a factor, present in crude extracts of orange juice, would decrease such fragility. The former (Scarborough 1940) stated that vitamin P did not control in any way the clinical symptoms of gross scurvy, but that it did produce an increased capillary strength in the scorbutic subject.

He found that the capillary strength of such subjects was not controlled by the administration of ascorbic acid, or of vitamins A, B_1, or D.

St.Rusznyak and Benko (1941) confirmed this concept of a distinct role of vitamin P in nutrition by feeding a scorbutogenic diet to rats. Because of vitamin C biosynthesis in rats, no scurvy resulted, but their capillary resistance diminished considerably in 5 - 6 weeks. The administration of 3 - 5 mg. of citrin per day restored the capillary resistance to normal in 10 - 14 days. In addition, Scarborough and Gilchrist (1944) stated that a statistical investigation of data obtained in 214 simultaneous determinations of capillary resistance and plasma ascorbic acid provided no support of the view that a low capillary resistance is associated with a deficiency of vitamin C. In fact, they found that lower, not higher, levels of capillary resistance were found in subjects with greater concentrations of ascorbic acid in the plasma.

Previously doubt had been cast on the finding of Bruckner and Szent-Gyorgyi that citrin consists of a mixture of hesperidin and eriodictin. Taking an increase in capillary resistance in man as a criterion of vitamin P activity, Scarborough (1941) found that both hesperidin and its aglycone, hesperitin, were active. However, they can only be given orally because of

their relative insolubility in aqueous solution of suitable pH. Their activity, classed as "moderate", was greatly exceeded by that of a preparation obtained from orange peel according to Szent-Gyorgyi's method. Scarborough, therefore, stated that this latter preparation contained at least two substances, neither of which has been identified.

Wawra and Webb (1942) prepared the pure chalcone of hesperidin, and showed that, as well as being soluble in water, it was effective in decreasing capillary fragility, and in preventing localized hemorrhages. The oxidation-reduction capacity of the hesperidin-hesperidin chalcone pair was also demonstrated. Higby (1943) offered evidence that hesperidin, in a soluble form, functioned as vitamin P and had a greater activity than hesperidin itself. The water-soluble forms included the sodium, calcium and other alkali and alkaline-earth salts, and the chalcone. He showed that the chalcone required methylation at position 6^1 to prevent its reversion to insoluble hesperidin.

One of the effects of excessive radiation has been shown to be an increase in capillary fragility. In 1947, Griffith et al stated that rutin appeared to hasten the recovery time in rats, after excessive irradiation. Rehers and Field (1948) showed that when dogs were given a midlethal dose of total-body X-irradiation,

the survival of rutin-treated to untreated dogs was in the ratio of 3:1. The surviving rutin-treated dogs were relatively free from petochiae and ecchymoses during the post radiation period and at autopsy. Clark et al (1948) fed guinea pigs a calcium flavonate preparation from lemon peel in their drinking water for a week before exposure to X-rays. The mprtality rate for animals thus treated was about one-half that for untreated animals exposed to the same amount of radiations, and the hemorrhagic symptoms in the surviving treated animals were less marked than in the untreated.

As a result of these findings, a distinct P-avitaminosis has been established, it being invariably associated with a much decreased capillary strength, and may be characterized by the development of spontaneous petechial hemorrhages, especially in areas exposed to stress. Thus, any material capable of increasing the strength of the capillary walls is said to have vitamin P activity. The chemical nature of many active extracts is still unknown, but research to date has attributed vitamin P activity variously to flavones, flavonones, flavonols and to some of their derivatives.

b) Biological Tests for Vitamin P Activity.

At the time that the strength of the capillary walls was thought to be dependent on vitamin C intake, Gothlin (1933) devised a test whereby it was possible to measure the strength of skin capillaries in humans. With the application of negative pressure to the hollow of the elbow, the number of petechiae found in a specific area, after a described length of time, and at either of two pressure levels, formed a basis for placing the patient into one of four categories of capillary strength. This was thought to constitute an indirect estimation of the vitamin C standard of the individual.

When capillary resistance was shown to be dependent, not on ascorbic acid, but on an active principle known as vitamin P, a biological test for vitamin P activity was devised by Bacharach, Coates and Middleton (1942). Negative pressure was applied to a shaven portion of the back of a guinea pig, and the Critical Petechial Pressure (C.P.P.) was noted at which petechiae were first seen. These workers found that when the results were plotted, with responses (C.P.P.) as ordinates and the log dose as abscissae, a given set of points fell on, or very close to, the regression line, calculated by the method of

least squares, for the vitamin P substance under test.

This biological test of Bacharach et al has caused some adverse comments because of the extraneous factors, such as changes in environmental temperature, or the presence of extravascular blood in any part of the body, which may affect the measurement, and because of the necessity for statistical analysis in the interpretation of the results. Bourne (1943) therefore used as a measure of capillary resistance, the negative pressure at which sufficient petechiae developed to create a uniform reddish-purple colour of the skin. A further test for vitamin P activity was devised by Majovski et al (1944) which was based on the resulting hemorrhage into the lungs when mice were placed in a jar from which the air was suddenly evacuated. However, this test was later shown to be non-specific by Raiman and Necheles (1947).

Doubt as to the practicability of all such biological tests for vitamin P activity may be expressed for the following reason. The blood content of the vascular bed is governed by the amount of circulating epinephrine, and thus the condition of the capillaries at the time negative pressures are applied will determine, to as great an extent as the strength of the capillary walls themselves, the resultant hemorrhage.

c) Physiological Function of Rutin and Other Vitamin P-Active Materials.

A flavonol glycoside, known as rutin, has become prominent in the literature because of its availability. Rutin was originally prepared commercially from flue-cured tobacco, but the low yield from an expensive raw material made necessary a search elsewhere for this product. It was first discovered by Schunck (1860) in buckwheat, and this plant has now become the common commercial source of rutin. Buckwheat leaves contain more of the material than other tissues of the plant, the content being greatest in the early blossoming stage.

Because of its close structural resemblance to hesperidin and eriodictin, rutin was immediately tested for vitamin P activity. After several years of clinical testing, rutin has now become established in human medicine as a remedy for weakened capillaries. Griffith, Couch and Lindauer (1944) fed rutin to 14 patients with hypertension, all of whom subsequently showed increased strength in the capillary walls. This work was confirmed by Shanno (1946). Ambrose and DeEds (1947) administered rutin to rabbits, and found that it decreased cutaneous capillary permeability.

The role of vitamin P-active substances as antioxidants is stressed in the literature. The French workers, Parrot, Lavollay and Galmiche (1944), using epi-catechin, have postulated that vitamin P exerts its various anti-hemorrhagic effects by the

inhibition of the oxidative destruction of circulating epinephrine. This hormone (epinephrine) then appears to decrease capillary fragility, capillary permeability and bleeding time through a vaso-constrictive or tonic action on precapillary blood vessels. Somogyi (1945) added vitamin C-free extracts of lemon juice filtrate residues, known to have a high vitamin P content, to solutions of crystalline synthetic ascorbic acid. He showed that this addition served to protect against the in vitro oxidation of vitamin C by the ascorbic acid oxidase of white cabbage, and other recognized oxidants of ascorbic acid, such as copper, the polyphenol oxidase of apples, and the peroxidase of horseraddish. Reder (1946) has reported that both fresh and previously boiled extracts of a number of vegetables exerted an inhibitory effect on the in vitro oxidation of ascorbic acid. Richardson et al (1947) have shown that flavonols, as illustrated by quercetin, quercitrin and rutin, are effective antioxidants for milk and lard. Furthermore, Wilson, Mortarotti and DeEds (1947) showed that rutin prolongs the action of epinephrine on intestinal strips, the prolongation presumably being due, they believe, to a protection of the epinephrine from oxidative destruction.

Histamine is known to dilate blood capillaries. The chief symptoms of histamine shock is a marked fall in blood pressure, apparently due to the great dilation of capillaries, with accompanying stagnation of blood in the tissues. Parrot and Richet (1945) showed that death from histamine could be prevented

by compounds closely related to flavonols. Wilson, Mortarotti and DeEds (1947) found that the administration of rutin intraperitoneally to guinea pigs decreased the mortality due to a lethal dose of histamine, if rutin precedes the histamine by 10 - 30 minutes. Simultaneous introduction, or at an interval of 45 - 60 minutes, did not decrease mortality. These workers stated that the protection may have been due to an increased level of epinephrine in the blood. Rutin, by an in vivo retardation of epinephrine destruction, would lead to an increase in its concentration, this epinephrine then having an opposing effect to that of histamine on the capillaries. However, Raiman, Later and Necheles (1947) found that rutin protected guinea pigs against the fatal effects of anaphylactic shock, but not against those of histamine. Levitan (1948) also stated that rutin had no effect in protecting normal rabbits from histamine shock. Wilson and DeEds (1948) contradicted this evidence, stating that to permit demonstration of the protective action of rutin on histamine shock, the amount of intravenously injected histamine is critical.

It is worth recalling that immediately after his isolation of citrin, Szent-Gyorgyi (1936) fed this material to guinea pigs on a scorbutogenic diet, and stated that the life of these animals was not only prolonged, but there was as well a marked decrease in the intensity of hemorrhages. He and his co-workers then suggested that experimental scurvy was caused by a combined lack of vitamins C

and P. This viewpoint was upheld by Elmby and Warburg (1937). However, Zilva (1937) questioned such a suggestion when he found that upon the administration of 1 mg. of citrin (2/3 mg. of hesperidin + 1/3 mg. eriodictin; or 1 mg. of purified hesperidin), the onset of scurvy was not delayed, nor was the fatal termination of the disease retarded in guinea pigs on a scorbutogenic diet. Detrick et al (1940) were in agreement with these results. Zilva also showed that sub-minimum prophylactic doses of ascorbic acid administered to such guinea pigs produced a pathological condition resembling that obtained by Szent-Gyorgyi and his colleagues by the administration of a daily dose of 1 mg. of citrin to these animals. Zilva explained the results of the Hungarian workers by postulating that their crude extract of citrin contained traces of ascorbic acid. When Szent-Gyorgyi et al failed to reproduce their earlier findings, the suggested role of vitamin P as a modifying factor in the scurvy syndrome was abandoned.

Recent work, however, indicates that there is a relationship of vitamin P-active materials to ascorbic acid. Cotereau et al (1948) showed that catechin, when fed to guinea pigs, permitted higher than average tissue storage of ascorbic acid than when the latter was supplied alone, even at a level of 10 mg. per day. Of more significance is the work of Ambrose and DeEds (1949) in which it was found that in guinea pigs receiving sub-minimum amounts of ascorbic acid, ascorbic acid plus rutin, and ascorbic acid plus quercetin,

the post mortem findings were essentially the same, except that the last two groups showed fewer fresh hemorrhages than the animals receiving ascorbic acid alone. They also stated that the combined supplements of ascorbic acid and rutin apparently prolonged the life of scorbutic guinea pigs.

The results of both groups of workers may be feasibly accounted for by the assumption that the two vitamin P-like materials were protecting the ascorbic acid from oxidative destruction. The work of Ambrose and DeEds (1949) shows the modifying effect of vitamin P-active materials on the scurvy syndrome, not directly as originally assumed by Szent-Gyorgyi, but indirectly through their sparing action on ascorbic acid. Thus, Szent-Gyorgyi's earlier work would be explainable if his crude extract did contain traces of ascorbic acid, as suggested by Zilva; while Zilva's findings would also be valid, since he fed purified hesperidin and eriodictin, with no vitamin C present.

d) Difficulties in Accepting Epinephrine Potentiation as Mode of Action of Vitamin P-Active Materials.

The mode of action of vitamin P-active materials has remained unsure. The theory advanced by the French workers (Parrot et al, 1944) that these materials protected circulating epinephrine from oxidative destruction, which hormone in turn decreased capillary fragility through a vasoconstrictive action on precapillary blood

vessels, was one that could satisfactorily explain many of the existing uncertainties in the knowledge of vitamin P-like substances. For instance, the reported biological tests for vitamin P activity would be valid under all conditions, since the actual measurement would be of the epinephrine concentration of the blood, this indirectly being an estimation of vitamin P activity. Furthermore, the beneficial use of vitamin P-active substances on the effects from X-irradiation and of histamine shock would be explained, keeping in mind the vasconstrictive action of epinephrine.

This theory of epinephrine potentiation as the mode of action of vitamin P-active materials can be adopted only with reservations at this time. Using a mammalian capillary bed preparation, Haley, Clark and Geissman (1947) estimated the relative activities of various topically applied vitamin P-like compounds themselves on microscopically visible vasometer responses in the blood vessels. Among the many such compounds found to be inert as vasoconstrictors were rutin and hesperidin, while the only highly active substances in that respect were the catechins. It should be borne in mind here that the previously mentioned theory of Parrot et al was arrived at with the use of catechins as their vitamin P-active substances. Clark and Geissman (1948) pointed out that vitamin P-like compounds required an ortho-dihydroxyphenolic structure with a side chain containing an unsaturation and a carboxyl group

in order to possess high activity in prolonging the effects of epinephrine. Furthermore, the ortho-hydroxy groupings must be free. They stated that epinephrine potentiating activity was probably related to the reducing powers of the compounds, quinone formation and metal-complexing or chelating capacity.

Clark and Geissman (1949) used both an intestinal segment assay and a spectrographic method (copper-catalyzed autoxidation of epinephrine in vitro) to study the potentiation of effects of epinephrine. Some 70 compounds were examined for the relation between molecular structure and activity of vitamin P-like substances in this epinephrine effect. The minimum molecular structure was found to be 3, 3^1, 4^1 - trihydroxyflavone. They also reported that the activity series obtained by the isolated smooth muscle assay method coincided neither with that by the spectro-photometric method, nor with capillary fragility-decreasing (vitamin P) activity as reported in the literature. Therefore, those workers stated that the theory is not supported that vitamin P-like substances act in the intact organism by inhibiting the oxidative destruction of epinephrine, and that the isolated intestinal segment response is not a valid assay method for vitamin P activity.

Thus, while a plausible theory does exist as to the mode of action of vitamin P-active compounds, considerable work remains to be done in order to make this theory, or a modification thereof, conclusive.

SPECIFIC OBJECT OF PROJECT

The results to date on the relationship of vitamin P-active materials to vitamin C have been of a predominantly qualitative nature. With the postulated antioxidant effect of rutin in mind, the specific object of this project was to determine the extent to which that substance would affect the apparent biological potency of vitamin C. The effect of rutin on ascorbic acid, both in its crystalline form and from a natural source, was examined. The odontoblast method of vitamin C bioassay was used as the criterion for assessing ascorbic acid potency.

EXPERIMENTAL PROCEDURE

This experiment was designed to test the extent to which rutin affected the apparent biological potency of synthetic ascorbic acid and of vitamin C from a natural source. The vitamin C carrier chosen for the test was commercial orange-grapefruit juice.

The odontoblast method of bioassay was used, and each animal was maintained on test for a 42-day period. Prior to the bioassay, as a basis for dosing, the orange-grapefruit juice was analyzed chemically for ascorbic acid, using both the dichlorophenol indophenol photometric method of Hochberg, Melnick and Oser (1943), and the 2,4-dinitrophenylhydrazine method of Roe and Oesterling (1944).

Healthy guinea pigs, weighing approximately 300 grams at the beginning of the assay period, were fed synthetic ascorbic acid or orange-grapefruit juice as the sole source of vitamin C. Rutin was fed to half the animals on each of these treatments.

Design of the Assay

The general design of this assay is shown in the allotment plan (Appendix Table I). Both sources of vitamin C were administered at four different levels of intake, these levels being 0.5, 0.79, 1.26 and 2.00 mg. ascorbic acid per animal per day. One hundred mg. of rutin were fed daily to half the animals on each assay material. The experiment was carried out in two replicates of 64 animals each. A total of 8 guinea pigs, 4 males and 4 females on each treatment,

was involved for the complete test. The animals were penned and fed in pairs, one pair of males and one of females per treatment in each replicate.

Animals

Guinea pigs from the stock colony at Macdonald College were weaned at 21 days of age, and after 7 ± 2 days from the weaning date, were randomized within sexes to each of the 16 feeding groups.

Diets

All animals received ad libitum the Macdonald College #6 Diet, plus water, throughout the 42-day test period.

Macdonald College Guinea Pig Diet #6

Oats	15.0%
Wheat	13.0
Beet pulp	20.0
Linseed Oilmeal	12.5
Skimmilk powder	15.0
Fishmeal	5.0
Brewers' dried yeast	10.0
Feeding molasses	5.0
Bone char	4.0
Salt (0.1% KI)	0.5

The diet was prepared by grinding, mixing, and pressing the mixture into pellets approximately one-eighth of an inch in diameter. Previous work in this laboratory has shown that the minor variations in consumption under ad libitum feeding, have no direct influence on this method of bioassay. Thus no feed records were kept.

Supplements

Vitamins A, D and E were administered weekly by a calibrated syringe. Each animal received 0.4 ml. of a mixture of ling liver oil, calciferol in oil and alpha tocopherol all dissolved in corn oil. The dose was such that each animal was given the equivalent of a daily intake of 3 mg. of alpha tocopherol, 425 i.u. of vitamin A, and 48 i.u. of vitamin D_2.

Sources of Vitamin C

The crystalline ascorbic acid was dissolved in distilled water immediately before dosing, while a fresh tin of orange-grapefruit juice was opened daily. Both these sources of vitamin C were administered directly by calibrated syringe at the four different levels of intake.

Administration of Rutin

Half the animals on each assay material were fed orally by calibrated tube approximately 100 mg. of crystalline rutin per pig per day.

Analysis of Experimental Data

At the conclusion of the 42-day feeding period, each animal was sacrificed. The incisor teeth were prepared for microscopic examination of the odontoblast cells, according to the procedure developed in this laboratory (Crampton 1947). The odontoblast cell measurements (microns) were analyzed statistically, with the variance

partitioned as shown in Appendix Table II. The method of Bliss and Marks (1939) was applied in the calculation of the relative potencies of the vitamin C sources.

RESULTS

Chemical

The average apparent vitamin C potencies, as determined by the two chemical methods on random samples of orange-grapefruit juice were

1) Method of Roe and Oesterling - 42.9 mg./100 ml.

2) Method of Hochberg, Melnick and Oser - 35.3 mg./100 ml.

The standard deviation of the individual determinations with the Roe and Oesterling method was ± 5.1%, and for that of Hochberg *et al* ± 3.5%.

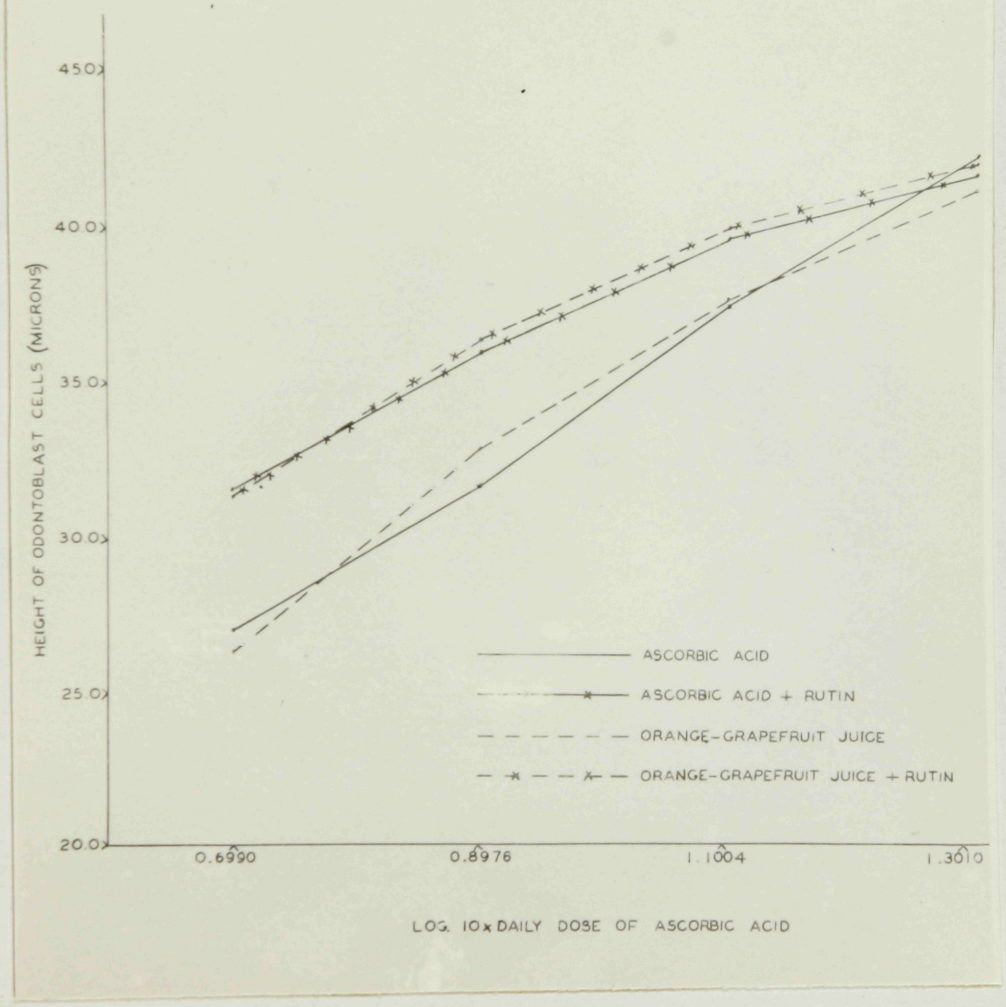
It has been found here (Macdonald College, unpublished data, 1948) that duplication of results between laboratories using either method was extremely difficult. The variability of data for vitamin C potency on identical samples was also very great. Because of the smaller variability of results, figures from the method of Hochberg *et al* were used in all calibrations, as well as in comparisons between biological and chemical values of vitamin C potency.

Biological

The mean heights of the odontoblast cells of the guinea pigs receiving synthetic ascorbic acid and orange-grapefruit juice, each with and without rutin, and at the four dosage levels of 0.5 mg., 0.79 mg., 1.26 mg., and 2.00 mg., are summarized in Appendix Table I. These results are illustrated graphically in Figure I.

FIGURE I

REGRESSION OF HEIGHT OF ODONTOBLAST CELLS ON DAILY INTAKE OF VITAMIN C FROM ASCORBIC ACID AND FROM ORANGE-GRAPEFRUIT JUICE; AND SHOWING THE EFFECT OF ADDED RUTIN.



The inclusion of the 2.00 mg. level of vitamin intake caused a deviation in the linear regression of odontoblast height on log. dose, and was therefore excluded from the analysis of variance (Appendix Table II). In determining the relative potency of orange-grapefruit juice in the absence of rutin, the 0.5 mg., 0.79 mg., and 1.26 mg., levels of vitamin intake were used. However, in the case of ascorbic acid plus rutin and orange-grapefruit juice plus rutin, the inclusion of the 1.26 mg. level resulted in a departure from parallelism, and accordingly only the two lower levels were used. (Bliss and Marks, 1939).

The potencies of ascorbic acid plus rutin, orange-grapefruit juice, and orange-grapefruit juice plus rutin were determined relative to synthetic ascorbic acid. These figures illustrating the effect of rutin, and the values of the mean ascorbic acid potencies of the orange-grapefruit juice, as determined chemically and biologically, are given in Table II.

Table II - The Vitamin C Potencies of Ascorbic Acid and Orange-Grapefruit Juice, Showing the Effect of Rutin

Material	Relative Potency*	Limits of Relative Potency P = 0.05	Biological Potency	Probable Limits of Biological Potency P = 0.05	Mean Vitamin C Potency - Chemical Hochberg et al.
	%	%	mg./ 100 ml.	mg./ 100 ml.	mg./ 100 ml.
Ascorbic Acid	100.0	99.0-101.0	35.0	34.6-35.4	-
Ascorbic Acid + Rutin	156.2	143.5-170.0	54.7	50.2-59.5	-
Orange-grapefruit juice	101.8	97.7-106.0	35.7	34.3-37.1	35.3
Orange-grapefruit juice + rutin	152.9	141.6-165.0	53.1	49.5-57.7	35.3

* Differences between Relative Potencies greater than 1.5 percentage units are statistically significant.

DISCUSSION

A close agreement between the mean vitamin C potencies as determined biologically and chemically (Hochberg, Melnick and Oser) was found for the orange-grapefruit juice. This agreement was in accordance with previous work in this laboratory.

Rutin, however, has presented a new and striking picture. Considering the apparent biological potency of synthetic ascorbic acid as 100%, the effect of supplementing this material with rutin was to increase its apparent biological potency by 56.2% at the 0.5 mg. and 0.79 mg. levels of vitamin intake. Furthermore, when orange-grapefruit juice constituted the source of vitamin C, rutin increased the apparent biological potency of the ascorbic acid therein by 48.8%, again at the 0.5 mg. and 0.79 mg. levels of vitamin intake. Thus rutin, a vitamin P-active substance, has,when administered with sub-optimum amounts of crystalline or naturally occurring vitamin C, a definite ability to increase the biological potency of ascorbic acid.

At levels of vitamin C intake above these, rutin showed no significant effect. Presumably at those levels, sufficient ascorbic acid was available to the animals, and the administration of rutin had little or no effect on the height of the odontoblast cells, which was, or was approaching a maximum.

The concept that rutin, and other vitamin P-like substances, may function in the body as antioxidants has been widely accepted. In this work it is postulated that rutin has delayed *in vivo* oxidative destruction of ingested ascorbic acid. The quantitative findings tend to support the qualitative findings of the relationship of vitamin P-active substances to vitamin C, as reported in the literature by Wilson, Mortarotti and DeEds (1947), Richardson *et al* (1947), Cotereau *et al* (1948), and Ambrose and DeEds (1949).

CONCLUSIONS

Rutin significantly increased the apparent biological value of ascorbic acid, when the vitamin was supplied in sub-maximum amounts, either in crystalline form or from a natural source.

The mechanism of action of rutin in this role is uncertain, but the prevention of *in vivo* oxidative destruction of vitamin C would explain these results.

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APPENDIX TABLES

APPENDIX TABLE I

Allotment Plan
Showing, 1) Distribution of 128 Animals to Treatment Groups,
2) Individual and Mean Heights of Odontoblast Cells (Microns).

Rutin Treatment	Ascorbic Acid per pig per day	Assay Materials: Ascorbic Acid Male	Ascorbic Acid Female	Ascorbic Acid Mean	Orange-Grapefruit Juice Male	Orange-Grapefruit Juice Female	Orange-Grapefruit Juice Mean
NIL	0.50 mg.	26.4 25.8 27.0 29.1	27.0 27.6 27.0 26.7	27.1	25.2 27.9 26.7 25.8	25.8 25.1 27.9 26.7	26.4
	0.79 mg.	31.8 30.9 32.4 32.7	32.7 30.6 32.7 30.9	31.8	32.1 31.5 32.7 33.0	31.8 30.3 35.1 37.9	33.0
	1.26 mg.	37.3 38.2 37.3 38.5	37.6 36.4 37.0 39.1	37.7	37.3 37.0 37.0 40.6	38.2 36.4 37.9 37.9	37.8
	2.00 mg.	42.4 41.5 42.4 43.0	43.0 41.5 42.7 42.4	42.4	41.8 42.4 39.1 40.6	42.7 44.2 40.9 40.2*	41.5
100 mg. Rutin per pig per day	0.50 mg.	31.2 32.1 30.9 31.8	32.1 33.0 31.5 30.9	31.7	32.7 31.2 32.4 31.8	33.3 31.8 27.9 30.6	31.5
	0.79 mg.	36.4 37.3 33.9 33.6	38.5 37.2* 34.2 37.3	36.1	36.4 37.6 34.5 37.3	36.7 37.3 37.3 35.1	36.5
	1.26 mg.	38.5 40.0 39.7 39.7	39.7 39.1 41.2 40.2*	39.8	39.1 39.1 41.2 40.3	40.3 41.2 39.1 40.6	40.1
	2.00 mg.	42.2** 40.6 42.1 40.3	42.4 40.9 44.2 42.4	41.9	42.1 44.2 40.0 42.1	42.4 42.7 42.7 42.1**	42.3

* Calculated value
** Corrected value.

APPENDIX TABLE II

Analysis of Variance of Mean Heights of Odontoblast Cells (Microns)

Source of variation	D.F.	$S(x-\bar{x})^2$	Variance s^2	Variance Ratio observed	Variance Ratio necessary minimum (P = 0.05)
Total	95	2001.95			
Sub Groups	23	1950.02			
Levels	2	1505.41	752.71	990.1	3.14
Rutin	1	316.49	316.49	416.5	3.99
Sex	1	0.97	0.97	1.3	3.99
Materials	1	1.05	1.05	1.4	3.99
Levels x Rutin	2	25.53	12.76	16.8	3.14
Levels x sex	2	0.69	0.35	0.5	3.14
Levels x materials	2	3.83	1.92	2.5	3.14
Rutin x sex	1	0.29	0.29	0.4	3.99
Rutin x materials	1	0.001	0.001	0.09	3.99
Sex x materials	1	0.36	0.36	0.5	3.99
Second Order Interactions	7	95.40	10.50	13.8	2.08
Third Order Interactions	2				
Calculated Values	4				
Error	68	51.93	0.76		

S.D. = 0.87