EFFECTS OF VITAMINS C AND P ON BLOOD SUGAR

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INTRODUCTION

Since its discovery three decades ago, ascorbic acid has been extensively studied, yet some of its physiological properties still remain for elucidation. One of these is the glycemic effect of the acid and its oxidized form, dehydroascorbic acid. Conflictng results have been reported on this point, and the species differences are by no means small (1).

In 1946 Stanly Levey first observed that ascorbic acid had increased the diabetogenic action of alloxan (2). Patterson in 1950 (3) found that large doses of dehydroascorbic acid caused permanent diabetes in rats but the diabetogenic effect of this form of vitamin C on rabbits was denied by the work of Banerjee et al. (4). The works of Fukuda (5), Fujita-Takesi (1) confirmed the observations of Stanly, and also found that large dose (250 mg/kg) was diabetogenic in the rabbit, and opposite findings were reported by Bera et al. in 1958 (6).

Closely associated with vitamin C therapeutically, the vitamin P-active substances (the Phenyl Benzo-r-Compound), at the beginning of their discoveries and designation, has been considered as one of the factors for the production of scurvy (7, 8), although this idea was abondoned by the investigations of Zilva (9) and others. They are now currently considered as antioxidants (10, 11, 12). Its potentiating effect on ascorbic acid has been noted when Crampton (10) found that foodstuffs had a higher biological value at the lower levels of vitamin C intake, than that shown by chemical assay. The results of further experimentatation on this point and many of the pharmacological properties of flavonoids especially rutin, have been reported repeatedly in the literature by Crampton (11) Wilson (12) Papageorge (13) William (14) Clark (15) Charles (16) Douglass (18) etc. The vitamin C-P relationship was briefly reviewed by Crampton (11) in 1950.

Reports of the glycemic effect of vitamin P-active substances were contradictory, William in 1949 (14) found that the compounds were not hyperglycemic, while Swift in 1952 (19) stated otherwise.

In the present paper, the authors tended to study the effect of vitamin C and P on blood sugar level and potentiating effect of vitamin P on the hyperglycemic effect of vitamin C.

MATERIALS & METHODS

Five young healthy, female albino rabbits were used. The weight of these rabbits at the beginning of the experiment was 1350-1600g. all the rabbits were fed with an ad libitum diet of sweet potatoes. The daily supplies to each rabbit were approximately 60g. of bean; 300g. of cabbage or rape; 50g. of carrot. The diet was constant throughout the experiment.

Instead of dividing the animals into groups, the experiment was conducted in three periods, each lasting for 8 to 13 days at intervals of one month or more. All the animals were used in each period, and the result so observed from the same individuals in different periods could be compared and

the bias introduced by individual variabilities was greatly minimized.

Because of its commercial availability and its relation abundance in the literature, rutin, was selected among the vitamin P-active substances in the experiment.

Since Crampton had reported that rutin had no potentiating effect on vitamin C for the odontoblast growth in guinea pigs when the vitamin C intake level reached 200mg/day (11), the authors wanted to know whether this is also true for diabetogenic action of the large dose of vitamin C, 250mg/kg vitamin C was used.

The three periods in the experiment were:

Period I (Control period); The animals were treated with intramuscularly injected 2.7ml/kg physiological saline on the first day of the experiment.

Period II (Vitamin C period); The animals were treated as in the period I, but 250 mg/kg vitamin C was used in place of saline.

Period III (Vitamin C and Rutin period): Same as in period II, but with an additional daily supply 100mg rutin per animal.

The blood sugar in each experiment was determined at the moment just prior to, and at 2, 4, 6, 8, hours and 1, 2, 3, to 12 days after intramusculay injection. Rutin in period III was administered at O hour on the first day, and on the days afterwards, 2 hours before the determination of blood sugar. Blood samples were collected at the marginal pinna vein, and its sugar content was estimated by Nelson's modification of Sonogyi's colourimetric method. (20.21). (In Period I and II, the colour intensity resulting from the treatment was measured by a visual colourimeter (Klett Biocolourimeter) (21) instead of photoelectric colourimeter as used in Period III (Fisher's Electrophotometer).

Vitamin C administered was in the form of sodium ascorbate prepared immediately before use by dissolving crystalline ascorbic acid in doubly distilled water and 2.5N sodium hydroxide was added so as to adjust the pH of the resulting solution to lay 5.5 to 5.9 (Herrick Index) (With U. S. P. specification, 5.6-7.6). 250mg of ascorbic acid dissolved in 2 ml. of water requiring 0.7 ml of 2.5 ml. sodium hydroxide for above pH.

Rutin used was crystalline powder. Since it is insoluble in water, a few drops of distilled water were added to the rutin powder and mixthoroughly, the paste of rutin was fed by force to the rabbits. To ensure that adequate amount of the drug was taken by the animal, 130 mg. of rutin was weighed out, the 30 mg. excess was considered as loss during the struggle of the animals when they were being fed.

RESULT

Table I The effect of ascorbic acid on blood sugar

Time	Blood sugar mg% Period I	Period II	Diff.	Percent. Change	Calculated t	P
0 hr	100.00±2.89	101.20±2.03	+ 1.02	+ 1.02	0.3240	>0.05
2 hrs	97.90±2.32	149.90±3.97	+52.20	+53.42	11.2186	<0.01
4 hrs	97.96±2.53	119.70±2.46	+21.74	+22.19	6.1598	<0.01
6 hrs	98.10±3.06	102.90±3.10	+ 4.80	+ 4.92	1.1170	>0.05
8 hrs	98.22±1.37	98.30±1.46	+ 0.02	+ 0.00	0.6021	>0.05
1 day	99.22±2.23	113.00±3.49	+13.78	+14.19	3.4320	<0.01
2 days	100.60±3.11	130.60±2.25	+33.34	+34.26	8.6814	<0.01

3 days	100.60±1.75	128.60±2.18	+28.00	+27.83	10.0260	<0.01
4 days	99.50±1.75	125.80±3.58	+26.30	+26.42	4.0934	<0.01
5 days	100.30±2.50	113.49±4.50	+13.10	+13.06	2.5413	<0.05>0.01
6 days	100.10±2.00	102.40±1.03	+ 2.03	+ 2.03	1.0222	>0.05
7 days	97.80±2.74	100.40±2.60	+ 2.60	+ 2.66	0.8324	>0.05

A. Effect of ascorbic acid on blood sugar: The effect of ascorbic acid on blood sugar is given in table I and Fig. I. The blood sugar was raised up to 53.42% higher at the 2nd hour in period II than the corresponding time in the control period. It dropped down to normal with 6 hours, and the hyperglycemic phase resumed gradually about 30% higher than normal till the fifth day.

B. Combined effects of ascorbic and rutin on blood sugar: From Table II the blood sugar estimated in period III was generally greater than that in period II, and the inital hyperglycemic phase had a longer duration, at the 6th hour, its deviation from the normal was still statistical significant (P 0.01) and from the lst day on, the hyperglycemia was maintained about for 5 days on the level of 22-42% higher. The difference between Period II and III is given in Table III. The peak value of the blood sugar at 2nd hour of period III was insignificantly differed from that of Period II with a more conservation statistical interpretation (Taking P: 0.01 as significance level), but with a lower precision, it is significant (Taking P: 0.05). For the rest of the days, the differennces between that of Period II were higher, but barely at the margin of significance.

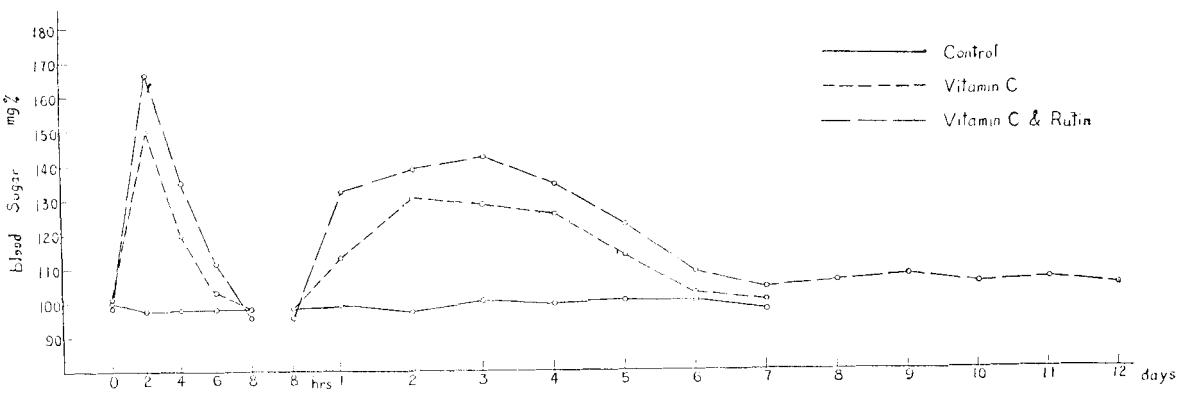

Fig. I Changes in blood sugar in three periods

Table II Combined effect of ascorbic acid and rutin on blood sugar

Time	Blood sugar mg% Period I	Period II	Diff.	Percent. Change	Calculated t	P
0 hr	100.00±2.89	98.60±2.53	− 1.40	− 1.40	0.3652	>0.05
2 hrs	97.70±2.32	166.25±4.63	+68.55	+70.16	13.0833	<0.05
4 hrs	97.96±2.53	135.14±2.71	+37.16	+37.95	14.3684	<0.05
6 hrs	98.30±3.06	111.22±2.83	+13.12	+13.37	4.5121	<0.05
8 hrs	98.22±1.37	95.58±3.55	− 2.64	+ 2.69	0.7195	>0.05
1 day	99.22±2.23	132.26±2.47	+33.04	+33.30	10.0229	<0.05
2 days	97.26±3.11	138.90±2.27	+41.64	+42.31	12.0972	<0.05
3 days	100.60±1.75	142.98±2.63	+42.06	+41.63	13.1581	<0.05
4 days	99.50±1.75	134.35±4.38	+38.35	+40.68	7.3864	<0.05

5 days	100.30±2.50	122.76±2.58	+22.46	+22.39	6.6549	<0.05
6 days	100.10±2.00	109.88±1.56	+ 9.78	+ 9.77	3.0431	<0,05
7 days	97.90±2.74	104.16±2.01	+ 6.36	+ 6.50	1.8294	>0.01

C. Effect of rutin on blood sugar: From Table III the blood sugar in Period III was no longer different from Period II from the 7th day on. Since the experiment was conducted for 12 days, the blood sugar level in the 7th to 12th day was considered as under the sole action of rutin. Then the blood sugar in Period I was considered a normal in the whole length of the period. After the comparsion by the method of student's "t", the result showed that difference was very significant. It is summarized below:

Treatment	Numbers of estimation	Degrees of freedom	Mean blood sugar mg%	Sum of squares
Rutin	60	59	105.728	419.91742
Normal	30	29	98.715	1404.7929
		Sum=88	Xdiff.=7.013	Sum=1896.6132

Pooled variance $=1896.61032/88=21.55239$

$S\bar{x}=\sqrt{S^2(n_1+n_2)/n_1n_2}=\sqrt{21.55239(60+30)/60\times30}=0.9785759$

$t=S\,x/\bar{x}=7.013/0.9785$ $\quad P<0.01$

Table III The difference between Periods II and III

Time	Blood sugar mg% Period II	Blood sugar mg% Period III	Diff.	Percent. Change	Calculated t	P
0 hr	101.20±2.03	98.60±2.53	− 2.60	− 2.57	0.7603	>0.05
2 hrs	149.90±3.97	166.25±4.63	+16.35	+10.91	2.6797	<0.05>0.01
4 hrs	119.70±2.46	135.14±2.71	+13.44	+12.90	4.2097	<0.01
6 hrs	102.90±3.10	111.22±2.03	+ 8.32	+ 8.09	2.1348	>0.05
8 hrs	98.30±1.46	95.58±3.55	− 2.72	− 2.77	0.7096	>0.05
1 day	113.00±3.49	132.26±2.47	+19.26	+17.04	4.5039	<0.01
2 days	130.60±2.25	138.90±2.27	+55.30	+ 4.06	2.5937	<0.05>0.01
3 days	128.60±2.18	142.68±2.68	+14.08	+10.95	4.0804	<0.01
4 days	125.80±3.58	134.35±4.38	+ 8.55	+ 6.80	1.6023	>0.05
5 days	113.40±4.50	132.76±2.58	+ 9.36	+ 8.25	1.8578	>0.05
6 days	102.40±1.03	109.88±1.46	+ 7.48	+ 7.30	4.1795	<0.01
7 days	100.40±2.01	104.16±2.01	+ 3.76	+ 3.75	1.3210	>0.05

It is noteworthy in the tables that when the blood sugar level increased, the S. E. M. had a larger value. This indicated that the individual variabilities were more pronounced when blood sugar raised by treatment.

DISCUSSION

From the data above ascorbic acid elevated the blood sugar up to 54% and followed by a immediately drop of blood sugar, then a lighter hyperglycemia for several days. There was a further elevation when rutin was added and rutin per se is hyperglyeemic. The pattern of changes in blood sugar in this experiment closely resembled to that of alloxan hyperglycemia. The inital hyperglycemia was due to

the destruction of beta cells in the islets of Langerhans and the drop of blood sugar level in the following days might be interpreted as due to the glyconeogenic effect of ascorbic acid (5) or the potentiating effect of ascorbic acid on the cortisone induced glyconeogensis (22). In association with this, ascorbic acid had been reported to proteolytic (23, 24).

In this paper in 1959, Fujita-Takesi (1) stated that ascorbic acid increased the degree of damage in the islets of Langerhans in alloxanized rabbits, but he did not show whether ascorbic acid per se was capable of damaging the beta cells in the islets. The beta cell damaging action of dehydroascorbic acid was found by Patterson (3) and MacDonald (25) in rats and its failure on rabbits was reported by Banerjee (14). The blood sugar change in the present experiment made the authors think that the ascorbic acid or its metabolic intermediate, dehydroascorbic acid exert a damaging action on the islets of Langerhans. But lacking a confirmatory conclusion in the literature and concieving evidences from experiments, the tentative idea above may be very probably erroneous.

The ideas regarding the relationship between ascorbic acid and adrenal cortical production and function were very convergent and contradictory. The presence of large amount of ascorbic acid in the adrenal and its depletion together with cholesterol in scurvy was interesting to most investigators. Hugher (27) accounted for the significance of the high concentration of ascorbic acid in this gland was that the acid might be essential in the production of the oxytype of the adrenocortical hormone which was in the main physiological responsibility for the maintanence of the increased blood sugar level (27), but in 1960 Perri-Golia (28) stated the opposite opinion that it inhibited the synthesis of adrenal steroids. Giroud in 1940 (29, 30) first reported that ascorbic acid wat necessary for the synthesis of corticosterone. Since that time, evidences for or against this moot question were abundant. The different opinions of the problem have been summarised with originality by Meikel John in 1953 (31). Observing the decreased excretion 17-ketosteroids in urine in scurvy, Bacchus in his series of reports stated that ascorbic acid was essential for the maintanence of cortical hormone from destruction (32, 33, 34, 35, 36) and this was generally agreed although taking 17-ketosteroids excretion as an index of adrenal activity was questioned by Banerjee in 1958 (37).

It has been reported that ascorbic acid decreased glucose utilization, (38) if it is true, this may be one of the roles in the ascorbic acid hyperglycemia, but it was denied by Bacchus in 1953 (34).

The antioxidant nature of rutin is a genrally accepted fact The potentiating. effect of the flavonoid, was chiefly investigated by Crampton. As to the hyperglycemic effect of rutin, it may be probably to its potentiating effect on the epinephrine (12, 14).

Since Swift reported that rutin had no demonstrable effect on blood sugar level of rabbits made diabetogenic by intravenous injection of alloxan (19), it is paradoxical to the author by the presence of a further raised blood sugar level with the administration of rutin in ascorbic acid hyperglycemia, which by the assumption of the author having a similar mechanism of action with the alloxan hyperglycemia. From the present experiments rutin per se was found to be hyperglycemic, therefore, the problem whether the blood sugar level maintained in the ascorbic acid and rutin treated animals was higher than the ascorbic acid treated alone is due to the cumulative effect of ascorbic acid or to potentiating effect of

rutin on the ascorbic acid hyperglycemia cannot be solved from the present experiments and further experimentations along the line are necessary for the solution.

SUMMARY

1. Induction of hyperglycemia in rabbits was demonstrated after intramuscular injection of large dose of ascorbic acid.
2. An elevation in the blood sugar in rabbits was noted after oral administration of large dose of rutin.
3. When both were administered, the hyperg-lycemia was accentuated.

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中 文 摘 要

維他命C及P對於血糖的影響

王慶襄 繆端生

維他命C可以提高血糖含量，如加用維他命P，則效應更顯著，且可保持更長之時間。作者認爲維他命P可使維他命C保持於體內，使發揮較久之作用。