EFFECT OF CHLOROPHYLL ON THE BIOLOGICAL ACTIVITY OF VITAMIN C

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Since the establishment of the chemical structure of chlorophyll (Chl) as tetrapyrrole compounds similar to hemin, many studies have been made concerning the effect of Chl on living organisms and it is now being used as a medicine, *etc.* Chl is also known to take part in the formation of vitamin C (VC) in vegetables, and, as Newbauer (1) pointed out, much Chl is found where much VC exists, suggesting the possible existence of some relations between Chl and VC also in animals. In order to investigate the effect of Chl on the biological activity of VC, an experiment was performed using guinea pigs as test animals. One of the authors (2) has established by experiment the effective minimum requirement of one guinea pig for VC to be 0.5 mg a day. The present authors tried to test the effect of Chl on the biological activity of VC and could confirm a definite promoting effect of Chl on the activity of VC.

EXPERIMENTAL

Test Animals and Feeds

The animals used in this experiment were guinea pigs of both sexes weighing about 250 g.

The basal feed consisted, in per cent, of bran 45, powdered oats 25 and dried milk (heated for 30 minutes at 120° to exclude VC) 30. At the same time two drops of cod-liver oil were given twice a week, and 1 g of powdered yeast once a week. For testing the effect following three chlorophyllin (Chln) preparations were employed, *i. e.*, Cu-Chln-Na, Mg-Chln-Na and Fe-Chln-Na. All these compounds, having 95–96 per cent purity, were prepared in the laboratory of Eizai K. K.

Method

The guinea pigs were divided into the following 13 groups, each consisting of 3 animals of both sexes. The 1st group: 0.5 mg L-ascorbic acid (AA) plus 1 mg Cu-Chln-Na. The 2nd: 0.5 mg AA plus 0.5 mg Cu-Chln-Na. The 3rd: 0.5 mg AA plus 5 mg Cu-Chln-Na. The 4th: 5 mg Cu-Chln-Na without

AA. The 5th: 10 mg Cu-Chln-Na without AA. The 6th: 0.5 mg AA plus 1 mg Mg-Chln-Na. The 7th: 0.5 mg AA plus 1 mg Fe-Chln-Na. The 8th: leaf vegetables, *Brassica chinensis* L. var. *Komatsuna* Matsum., containing 0.5 mg AA. The 9th: bean sprouts containing 0.5 mg AA. The 10th: 0.5 mg AA plus ferric citrate. The 11th: 0.5 mg AA added with CuSO₄. The 12th: AA 1 mg. The 13th: AA 0.5 mg. Each animal was placed in a wire cylinder, 27 × 30 cm, straws being spread on the bottom. The straws were renewed every three days.

As the preparatory experiment each guinea pig was fed the mixture of bran and bean curd lees "tofu", gradually adding increasing amounts of the basal feed and weeds and decreasing the former mixture till it was entirely omitted. When the animals were accustomed to the basal feed, weeds were excluded. Then the experiment started by feeding the basal feed and water together with the test food, and each animal was weighed once a day at a definite time.

The test foods were prepared as follows: In AA groups, 3 drops of aqueous AA solution containing 0.5 or 1 mg were given. In Chln groups, 0.5 mg AA and 0.5, 1 and 5 mg, respectively, of Cu-Chln-Na were given. Other groups received 1 mg Mg-Chln-Na and 1 mg Fe-Chln-Na. The 4th group received 5 mg Cu-Chln-Na alone and the 5th 10 mg Cu-Chln-Na. Only the leaves of the greens and the stalks of bean sprouts were given to the 8th and 9th groups, each containing 0.5 mg AA. The Fe group (10th) received 0.389 mg ferric citrate containing the amount of Fe contained in 1 mg Fe-Chln-Na together with 0.5 mg AA; and Cu group (11th), 0.365 mg CuSO₄ containing the amount of Cu corresponding to 1 mg Cu-Chln-Na in addition to 0.5 mg AA. All these chemicals were given as a solution using phials.

The experiment consisted of 7 periods. The 1st period was from May to July, 1951. The 2nd, from October to November, 1951. The 3rd, from September to November, 1952. The 4th, from May to July, 1953. The 5th, from September to November, 1953. The 6th and 7th, from April to June, 1954. Each period lasted 40–60 days, during which appetite, weights, motion and appearances of the animals were observed.

Results

The results of the experiment as given in Table I, as well as Fig. 1 and 2 show that 0.5 mg AA group (13th) gained on an average only 0.7 g in weight per day, while those groups receiving 1 mg each of Cu-Chln-Na, Mg-Chln-Na and Fe-Chln-Na respectively gained 3.6, 3.2 and 2.9 g indicating far more beneficial growth and apparent health of the groups of Chln addition than the group without addition. Moreover, those groups receiving 1 or 5 mg Cu-Chln-Na and 1 mg Mg-Chln-Na together with 0.5 mg AA gained almost as much as 1 mg AA group (12th). The group receiving 5 mg Cu-Chln-Na together with 0.5 mg AA (3rd) gained 3.3 g per day, a somewhat less weight gain than the group receiving 1 mg Cu-Chln-Na (1st). No remarkable effect corresponding to a five-fold amount of Cu-Chln-Na was observed. The excrements of the 1st group were light brown in color while those of the 3rd one were dark green, possibly the excess Chln being excreted undigested.

TABLE I
Effect of Chlorophyllin on the Growth of Guinea Pigs.

Group	Treatment	Initial average weight	Average weight gain per day
		g	g
1	AA 0.5 mg + Cu-Chln-Na 1 mg	220.5	3.6
2	AA 0.5 mg + Cu-Chln-Na 0.5 mg	248.3	2.3
3	AA 0.5 mg + Cu-Chln-Na 5 mg	252.3	3.3
4	Cu-Chln-Na 5 mg	269.7	-0.4
5	Cu-Chln-Na 10 mg	251.3	0.9
6	AA 0.5 mg + Mg-Chln-Na 1 mg	267.8	3.2
7	AA 0.5 mg + Fe-Chln-Na 1 mg	254.5	2.9
8	Komatsuna containing AA 0.5 mg	265.0	2.7
9	Bean sprouts containing AA 0.5 mg	216.1	1.8
10	AA 0.5 mg + $\text{FeC}_6\text{H}_5\text{O}_7$ 0.389 mg	264.3	2.1
11	AA 0.5 mg + CuSO_4 0.365 mg	260.0	1.7
12	AA 1 mg	309.3	3.8
13	AA 0.5 mg	238.0	0.7

FIG. 1 *Animal Experiment Using Chlorophyllin Preparations together with Ascorbic Acid.*

The group receiving 0.5 mg Cu-Chln-Na (2nd) gained 2.3 g per day, being less than the 1st group. The groups receiving 5 or 10 mg Cu-Chln-Na alone began to lose weight after 16–18 days, without appetite, and too inactive to move. After feeding 2 mg AA, they became suddenly active and gained weight. The group fed green leaves (8th) gained 2.7 g, more marked than 0.5 mg AA group (13th), but less than the 1st group receiving 0.5 mg AA plus 1 mg Cu-Chln-Na. This suggests that green leaves containing 0.5 mg AA may possess less than 1 mg Chln.

The bean sprouts group (9th) containing no Chl gained more in weight

FIG. 2 *Animal Experiment with Ascorbic Acid.*

than 0.5 mg AA group (13 th), though less than the groups receiving Chln or green leaves, possibly suggesting that vegetables have other supplementary components which are more effective than synthetic Chln. It is worthy of note that these vegetables (green leaves and sprouts) were eaten at once by the animals as soon as they were given.

The effects of inorganic salts which were contained in Chln preparations, *i. e.*, 88.6 γ Fe and 144.7 γ Cu respectively, were tested as $\text{FeC}_6\text{H}_5\text{O}_7$ and CuSO_4 but it proved to be less effective than Chl (Table I).

SUMMARY

1. The effect of Chl on the biological activity of vitamin C was tested using guinea pigs and it was proved that the animals receiving 0.5 mg ascorbic acid (minimum requirement) together with 1 mg Chl, showed the effect corresponding to those receiving 1 mg ascorbic acid alone.

2. Administration of Chl alone even in a large amount could never prevent vitamin C deficiency.

3. When vitamin C is administered as natural vegetables, the effect of Chl plus vitamin C (green leaves) proved to be more effective in gaining body weight than that of the vitamin alone (bean sprouts).

REFERENCES

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2. Inagaki, Ch., *Vitamin C in Natural Vegetables* (Japanese). p. 79, Sangyo-Tosho, Tokyo, (1948).