

# CCXCV. INTERRELATIONS OF THE VITAMINS

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In recent years the possibility of an antagonism between vitamins A and C has often been mentioned. This antagonism, if it exists, might be revealed by the partial inhibition of the effects of vitamin C in scurvy by the simultaneous administration of vitamin A.

During the last few years we have had the opportunity of making a close study of vitamins A, B complex, C and D, and being somewhat sceptical about the existence of the above-mentioned antagonism we decided to make a thorough investigation of the subject. We wish to make it clear that when we speak of antagonism we have in mind harmful antagonism.

It is generally agreed that a balance should exist between the various vitamins. A state of equilibrium can be preserved in spite of considerable variations in the amounts of the vitamins supplied, but if one particular vitamin is present in too great an excess, the symptoms of hypervitaminosis will be the consequence. In such cases it may be possible to restore the health of the organism by supplying an adequate quantity of one or more of the other vitamins. The equilibrium between the vitamins is now re-established by the increased supply of the relatively deficient vitamins, an example of beneficial antagonism.

The existence of beneficial antagonism between the fat-soluble vitamins A and D on the one hand, and the water-soluble vitamins B and C on the other, appears to be unquestioned. Publications by workers such as Hopkins [1923], Harris & Moore [1928], von Euler [1933], Höjer [1926] and Light *et al.* [1929], reveal clearly its existence. We have therefore considered it superfluous to investigate this problem and have limited ourselves to a study of the existence of the possible harmful antagonistic action of vitamins A and D on vitamin C.

In studying the available literature in order to establish the grounds for the belief that there is a harmful antagonism between vitamins A and C, we have succeeded in finding only one publication which in our opinion seems to be reliable and which might be construed as revealing such an antagonism. This publication is by Mouriquand & Michel [1922], but even here there is no question of an actual antagonism between vitamins A and C, because these workers rightly limit themselves to the demonstration that cod liver oil under given conditions may have a destructive action on the antiscorbutic effect of vitamin C. We have therefore come to the conclusion that there is no evidence for such antagonism in the case of either man or the lower animals. On the other hand, there is evidence in favour of the existence of a synergistic action between these vitamins. Thus we might mention innumerable cases in which physicians prescribe cod liver oil and orange juice for infants and also the unquestionably good results obtained with various commercial preparations containing vitamins A and C.

## EXPERIMENTAL

Our experiments were carried out with a liquid "Preparation X", composed chiefly of a concentrate of vitamins A and D, malt extract, orange juice, sugar and water. 1 ml. of this product (=1.32 g.) contained 800 I.U. vitamin A (i.e. 600 I.U. per g.), 120 I.U. vitamin D, between 5 and 5.5 I.U. vitamin C and also a little of the vitamin B complex. The ratio of vitamin A to vitamin C was therefore 800 to 5.3 or 150 to 1 in terms of International Units. Preparation X contained about 1% of the above-mentioned vitamin concentrate.

Three series of preliminary experiments were made; in each series there were three groups of guinea-pigs, with four animals in each group.

The main object was to compare "Preparation X", which contained vitamins A and D as well as vitamin C, with "Preparation Y" which differed from Preparation X only in the fact that it contained no vitamin A or D. It was desired to ascertain whether vitamins A and D in Preparation X would inhibit the action of the vitamin C which it contained.

The daily diet in the three groups is shown in Table I.

Diet	Added vitamins	Ratio vitamin A : vitamin C
(1) Basal diet + 4 g. Prep. X	2400 I.U. vitamin A + 16 I.U. vitamin C	150 : 1
(2) Basal diet + 4 g. Prep. Y	23 I.U. vitamin A + 16 I.U. vitamin C	1.43 : 1
(3) Basal diet alone	No vitamin C	—

The basal diet was that of Tillmans and consisted of oats, milk, hay and a little cod liver oil, equivalent to a dose of 23 I.U. vitamin A and 3 I.U. vitamin D per diem. Preparations X and Y were given mixed in milk.

The animals in Groups 1 and 2 were fed on the basal diet alone until increase in weight ceased or loss of weight took place and were then given the preparations. Weights were recorded twice a week over a period of 50 days except in the case of Group 3 when the death of the animals occurred before the close of the testing period.

Following the preliminary experiments in which only a small number of animals was used in each group, work was carried out with a series composed of four groups with 10 animals in each group.

In order to compare the action of cod liver oil with that of Preparation X an extra group was included in this series. The animals in this extra group received the same quantities of vitamins A, D and C as those of the group receiving Preparation X, but vitamins A and D were given in the form of cod liver oil. Vitamin C was administered in the same way as in Group 2, namely in Preparation Y. Another reason for introducing this extra group fed on cod liver oil was to verify the experiments of Mouriquand & Michel [1922] in which they had used a basal diet with added orange juice and cod liver oil.

In order to have the same quantity of vitamin A (2400 I.U. vitamin A) in proportion to vitamin C as in the group fed on Preparation X, and so that the quantities of cod liver oil and of Preparation X should be of the same magnitude, we used 4 g. cod liver oil containing 600 I.U. vitamin A per g. The basal diet was the same as before. The cod liver oil was converted into an emulsion and mixed with the milk given to the animals.

The action of the vitamin C administered was assessed on the one hand by the increases in weight and the general condition and appearance of the animals, and on the other hand by post-mortem examinations of the animals which died or were killed and by X-ray photographs of the thorax.

We have prepared graphs showing the mean variations in weight for the groups of animals in each experiment, that is to say for the Preparation X group, Preparation Y group, and the negative control group in each of the three experiments of the preliminary series (see Figs. 1, 2 and 3) and for the Preparation X group, Preparation Y group, Y with cod liver oil group and the negative control group in the larger-scale experiment (see Fig. 4).

One animal fed on Preparation Y died from pneumonia. Three of the animals receiving cod liver oil died, probably from scurvy (post-mortem examination revealed scorbutic signs in each case). The negative control animals all died in less than 50 days with typical symptoms of scurvy.

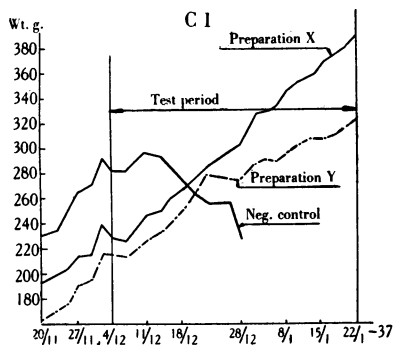


Fig. 1.

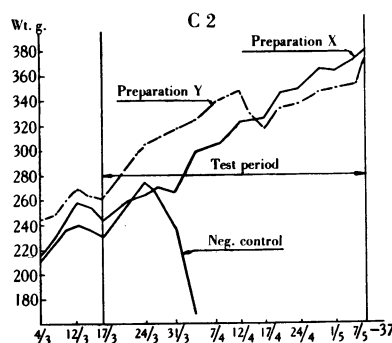


Fig. 2.

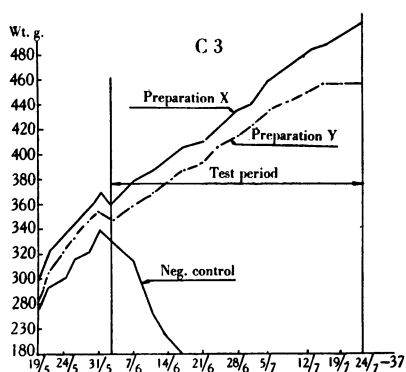


Fig. 3.

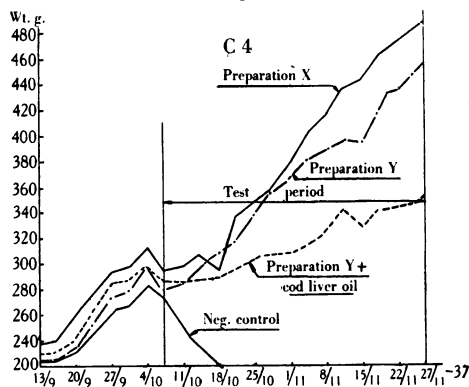


Fig. 4.

As can be seen from the curves, the mean increase in weight was in every series a little greater for animals receiving Preparation X (including vitamins A and D) than for those receiving Preparation Y (without added vitamins A and D). If, then, increase in weight is taken as a criterion of the effect of vitamins A and D on the action of vitamin C, the results of every experiment seem clearly to indicate that there is no antagonism between vitamins A and D and vitamin C. It will be noted that the weight increase of the animals receiving cod liver oil (Fig. 4), was less than that of those animals receiving Preparation X or Y.

In spite of the very large amount of vitamin A administered, large both from an absolute point of view and in proportion to the amount of vitamin C,

hypervitaminosis-A did not occur and the vitamin A appears to have reinforced the action of vitamin C.

The general appearance of the animals of the X groups was excellent, that of the Y groups very good, that of the cod liver oil group bad and the animals of the negative control groups were of course miserable in appearance.

#### *Results of the autopsies*

After the animals had died or had been killed at the end of the testing period, observations were made regarding the existence of haemorrhages and fragility of the bones and the condition of the thorax was examined. The results of these examinations are given below (Table II).

Table II. *Results of autopsies*

Series no.	Group no.	Preparation	Haemorrhages	Bone fragility	Scorbutic rosary
I	1	X	None	Normal	None
	2	Y	Almost none	Normal	Traces
	3	Negative control	Severe	Very fragile	Pronounced
II			Exactly as for Series I		
III			Exactly as for Series I		
IV	1	X	None	Normal	None
	2	Y	Almost none	Normal	Traces
	3	Y + cod liver oil	Rather pronounced	Rather fragile	Rather pronounced
	4	Negative control	Severe	Very fragile	Pronounced

All the animals in Group 1 were plump and healthy and the carcasses were light in colour.

The animals belonging to Group 2 (Preparation Y) were likewise in good condition, but they showed a few small haemorrhages, the colour of their carcasses was not so light as in Group 1, neither were they quite so plump. The bones were strong. X-ray examination of the thorax did not give quite so favourable a picture as in the case of Group 1. It could not be said that animals belonging to Group 2 exhibited distinct scorbutic symptoms and the difference between those receiving Preparation X and those receiving Preparation Y was very slight on the average. Any difference that existed was in favour of the "X" animals in Group 1.

All the negative control animals (Group 3 in the preliminary series I, II and III and Group 4 in the "Large Series" IV) showed typical symptoms of scurvy, severe haemorrhages, fragile bones (sometimes even breaking before death) and a pronounced scorbutic rosary.

Animals belonging to Group 3 in the "Large series" (cod liver oil) also showed symptoms resembling those of scurvy.

Mouriquand & Michel [1922] have shown that cod liver oil, under the conditions obtained in trials with guinea-pigs, can inhibit the action of ascorbic acid. However, we agree fully with the reservations made by them that one cannot conclude from these experiments on guinea-pigs that cod liver oil given in ordinary doses inhibits the antiscorbutic action of vitamin C in man. In this connexion it is interesting to note that Wieland [1936] denies the existence of any antagonism between vitamins A and C in human beings. It must be remembered that the doses of cod liver oil administered to the guinea-pigs in our experiments were extremely large. Taking into consideration the relative weights of the animals and man, the corresponding dose for a man would amount to nearly 1 kg. cod liver oil a day.

*Stability of vitamin C in presence of vitamins A and D*

It has been claimed that vitamin C may be destroyed by vitamin A *in vitro*. We have examined the chemical relations between vitamins A and D on the one hand and vitamin C on the other, using emulsions corresponding in their make-up to Preparations X and Y. The results of such investigations will not be given in detail on this occasion but they showed that vitamin C was not appreciably reduced in amount in either preparation 3 months after the date of their manufacture. After an interval of 6 months the diminution was 10–15%. These tests were made with a commercial preparation and no special precautions had been taken to prevent the destruction of vitamins. The analyses were made by Tillmann's method. Thus it was impossible to prove that vitamin C had been affected by the presence of vitamins A and D in the preparation.

## SUMMARY

1. Using guinea-pigs as experimental animals it was found that vitamins A and D had no antagonistic action on vitamin C, even if the doses of the fat-soluble vitamins were very large in relation to the dose of vitamin C.
2. It seems possible that vitamins A and D can reinforce the action of vitamin C, even if the excess of vitamins A and D over vitamin C is very considerable.
3. Under the conditions of our *in vitro* experiments vitamins A and D had no destructive chemical action on vitamin C.
4. Very large doses of cod liver oil inhibited the antiscorbutic action of vitamin C in guinea-pigs.

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