I. THE EFFECT OF EXCESS OF VITAMINS A, B₁ AND C ON THE ASSAY OF VITAMIN D AND OF EXCESS VITAMIN D ON THE ASSAY OF VITAMIN A

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SEVERAL workers have found that the response to a particular dose or to a graded series of doses of one dietary factor is influenced by the amount of another dietary factor given at the same time; e.g. the response to doses of vitamin D when small amounts of mineral phosphates were added to the diet [Bruce & Callow, 1934], the weight response to vitamin D when different amounts of vitamin A were given daily [Coward *et al.* 1932], the weight response to vitamin B₁ when different amounts of autoclaved yeast as a source of vitamins B other than B₁, were included in the basal diet [Coward *et al.* 1933]. Whether these results be interpreted as an "interdependence" of the vitamins or as a "sparing action" of one vitamin on another, it is clear that every factor known to be essential except the one under investigation must be supplied in amounts adequate for all responses whenever an estimation of potency is to be made. Otherwise, in comparisons of a food substance which contains certain quantities of different vitamins with an international standard that contains only one, the vitamins of the food substance may make good the partial deficiencies in the basal diet and thus bring about a bigger response than would be brought about by the one vitamin alone which is being determined. This was also suggested by Bacharach *et al.* [1931], who showed, however, that small additions of carrot, 1 g. daily, to the Steenbock 2965 ration had no influence on the rate of growth of the rats or on the development of rickets with and without doses of vitamin D.

The experiments mentioned above dealt with the influence of partial deficiencies in the basal diet. The influence of an excess of any one vitamin on the determination of another vitamin has been investigated in the experiments described in this paper.

I. *The influence of large doses of vitamins A, B₁ and C respectively on the response of rats to a dose of vitamin D as measured by the line test*

The tests were carried out according to the line test technique on young rats described by Dyer [1931]. All the rats were given a single dose of international standard vitamin D on the first day of test and in addition one half of the rats received a daily supplement of the vitamin under examination for the first 9 days. The animals were killed after 10 days and the line test applied to the distal ends of the ulnae and radii.

The supplements were as follows:

Vitamin A. A solution of carotene (B.D.H.) in coconut oil of such strength that 20 mg. solution contained 20 γ carotene was used. The doses of 40 γ carotene per rat per day were administered by drops directly into the animal's mouth from a calibrated dropping tube at 40–45° (Table I, exp. 1–4).

Vitamin B₁. A vitamin B₁ adsorption product on acid clay, containing 100 i.u. vitamin B₁ per g. was used. The doses of 10 or 15 i.u. were weighed daily in separate portions and moistened with a little water to prevent scattering of the powder. No instance of an animal refusing to eat its dose occurred (Table I, exp. 5).

Vitamin C. A solution of ascorbic acid in distilled water was prepared daily immediately before use and doses of 0.15 mg. were delivered directly into the animal's mouth from a micrometer syringe (Table I, exps. 6 and 7). This test was included in spite of the generally accepted fact that the rat does not need a supply of vitamin C in the diet.

The results are given in Table I.

Table I. *The effects of vitamins A, B₁ and C respectively on the response of rats to a dose of vitamin D*

1	2	3	4	5		6
Exp.	Daily dose of supplementary vitamin given to one member of each pair of rats for 9 days	Dose of vitamin D given to all rats on the 1st day of test i.u.	No. of pairs of rats	Average healing of the group of rats		Apparent dose ratio vitamin D + supplement vitamin D alone
				Vitamin D + supplement	Vitamin D alone	
1	40γ carotene	10	11	1.64	2.05	0.74
2	"	5	15	2.07	1.93	1.10
3	"	5	15	2.07	1.80	1.21
4	"	5	16	1.60	1.66	0.95
5	10 or 15 i.u. vit. B ₁ 100 units in 9 days	10	12	2.68	2.50	1.14
6	0.15 mg. as- corbic acid	10	12	2.59	2.17	1.34
7	"	5	20	2.50	2.28	1.17

In col. 5 of Table I is given the average healing brought about by the dose of vitamin D plus the supplement and that brought about by the dose of vitamin D alone in each experiment. Since the relation between the amount of healing and dose of vitamin D given is not a straight line but a logarithmic curve, each ratio for healing can, by means of this curve, be converted into the apparent dose ratio. This ratio is given in col. 6.

In view of the known variation in all biological responses and the relatively small numbers of animals used, none of these ratios can be considered significantly different from unity. It must be concluded that very large doses of vitamins A, B₁ and C respectively have no influence on the amount of healing brought about by a small dose of vitamin D. It is therefore unnecessary, in determinations of the vitamin D content of a substance, to take into consideration the amounts of these vitamins likely to be present in that substance.

II. *The influence of a large dose of vitamin D on the weight response of rats to a dose of vitamin A*

The technique used in the vitamin A test has been fully described by Coward *et al.* [1930]. Young rats of about 30 g. weight and 18–21 days old were given the vitamin A-free diet immediately after weaning. The sexes were segregated and groups of about 7 rats of the same sex were kept together in a cage. The young rats were weighed at first weekly and after the first 4 weeks twice weekly, until

they ceased to grow and showed a loss in weight. As the animals became ready for dosing each rat was put into a separate cage. Three groups were formed. The litters were divided as equally as possible among the three groups but precedence was given to the date of depletion rather than to the genetic constitution so that all the rats ready on any one day were divided equally among the three groups.

During the depletion period, vitamin D was supplied as a solution of irradiated ergosterol in olive oil of such strength that 8 I.U. vitamin D were contained in 20 mg. solution. A single drop of this solution was fed to each rat once a week. This had been shown to be an ample allowance for rats receiving the vitamin A-free diet [Coward *et al.* 1932].

When the rats became steady in weight they were given doses of vitamin A twice a week (three doses on one day and four doses 3 days later [Coward & Key, 1934]. In addition they were given a single dose of vitamin D per week, as during the depletion period, containing the following amounts: Group 1, 8; Group 2, 25; Group 3, 100 I.U.

The vitamin D used during the test period was a solution of calciferol in olive oil of such strength that the required weekly dose was contained in 20 mg. solution.

The dose of vitamin A chosen was 2 mg. daily of a particular sample of cod liver oil, known in this laboratory as "oil Z". The dose was small so that either a depressing or an enhancing effect of the excessive doses of vitamin D could be detected. The oil contains approximately 1500 I.U. vitamin A and 100 I.U. vitamin D per g. The rats were therefore receiving a daily dose of 3 I.U. vitamin A. At the end of the first week the dose was reduced to 1 mg. cod liver oil, i.e. to 1.5 I.U. vitamin A per day. The rats would also receive from the cod liver oil 1.4 I.U. vitamin D during the first week and 0.7 I.U. vitamin D per week for the remaining 4 weeks of test. The animals were weighed once a week during the test period. Table II shows the average increases in weight of the three groups of rats.

Table II. *Growth response to a dose of vitamin A with variations in the vitamin D supplied*

Dose of vitamin D in the basal ration. I.U. per week	No. of rats		Total increase in wt. (g.)		Total increase in weight during 5 weeks g.	Mean increase in weight per rat per week g.
			1st week	2nd-5th weeks		
	♂	♀	2 mg. C.L.O. oil Z daily	1 mg. C.L.O. oil Z daily		
8	4		8	68	76	3.8
		10	39	142	181	3.6
25	6		12	96	108	3.6
		11	26	170	196	3.6
100	4		20	60	80	4.0
		10	33	191	224	4.5

There is evidently no difference in response with the three levels of vitamin D intake and it may be concluded that giving a large excess of vitamin D, even twelve times the amount which had been considered ample, had no influence on the weight response of a group of rats to a dose of vitamin A during a 5 weeks' feeding period.

Although previous workers have shown that the effect of a small dose of a vitamin may be diminished by a partial deficiency of another vitamin in the diet

given during the test, the effect of the small dose cannot be enhanced indefinitely by giving more and more of the other vitamins. Thus there is no "sparing action" in the influence of one vitamin on another. Further, excessive amounts of one vitamin in the diet do not diminish the response to a small dose of another vitamin. There is, therefore no danger of the determination of one vitamin in a substance being spoilt by the presence of even excessive amounts of other vitamins.

SUMMARY

1. The response to a small dose of vitamin D (as measured by the line test) was not influenced by giving at the same time excessive doses of vitamins A, B₁ or C.

2. The weight response to a small dose of vitamin A was not influenced by giving a graded series of excessive doses of vitamin D.

3. In assaying vitamin A or vitamin D, it is unnecessary to consider the possible presence of another vitamin in the substance under test, provided that the basal diet is adequate.

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