THE ANTIVITAMIN D FACTOR IN ROUGHAGES [1])

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SUMMARY

A method has been worked out to separate the vitamin D in grass and hay from the rachitogenic factor. In this way it is possible to determine the true vitamin D content of roughages. The traditional method used till now gives the physiological antirachitic activity, which is often less than the activity corresponding to the real vitamin D content. In some hay samples we found that this physiological antirachitic activity corresponded with the real vitamin D content owing to the absence of the rachitogenic factor, whereas in artificially dried grass no such cases were found.

This rachitogenic factor is not a digitonisable phytosterol and is present in that fraction, prepared by chromatography, which contains alpha- and beta-carotene. Recently it has been announced that the factor is carotene itself. The results now reported support this view, but the evidence is not yet complete.

INTRODUCTION

The existence of an antivitamin D factor in green feeds, artificially dried grass and hay has been reported recently. (GRANT, 1951, WEITS, 1952). This implies that previous vitamin D determinations on such feeding stuffs are, in fact, a function of the total vitamin D activity and the rachitogenic activity. There is some evidence that the antirachitic factor is active for the ruminant as well as for the rat (EWER, 1948–1949). If this can be established then vitamin D activities as normally determined are sufficient for practical purposes. Nevertheless, it is of practical and scientific interest to obtain more information about the rachitogenic factor and to determine to what extent it depresses the vitamin D activity of roughages.

Experiments have therefore been carried out to find a method for the separation of vitamin D from the rachitogenic factor and thus to determine the true vitamine D content of roughages.

EXPERIMENTAL

Chromatografic adsorption was used to separate vitamin D from the rachitogenic factor. The choice of adsorbent and eluting agents was made so that the fraction in which vitamin D was present could be predicted. Confirmatory tests were done with pure calciferol. As nothing was known as to the probable behaviour of the rachitogenic factor, initially the unsaponifiable residue of the grass-fat was arbitrarily divided into five fractions each containing several carotenoids. By feeding each fraction dissolved in arachis oil, supplemented with a known amount of vitamin D, to groups of rachitic rats it was possible to check that separation of vitamin D and the rachitogenic factor had been achieved and also to determine which fractions contained each of these factors. After several experiments it became clear that three of the five fractions had no rachitogenic effect. Ultimately no more vitamin D was given to that group of rats receiving the vitamin D containing fraction of the grassfat. It was, therefore, possible to carry out direct vitamin D determinations in the chro-

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matographed grassfats and thus in the grass samples from which they originated. Some results are shown in Table 1.

Table 1. The influence of fractions of grassfat on pure vitamin D.

Exp.	Daily dose			X-ray healing	Percent recovery
A	Calciferol		0.15 I.U.	1.83	
	„		0.30 „	3.70	
	„		0.60 „	5.35	
	Fraction 1 *) (carotene)	+	0.30 „	1.80	49
	„ 2 **) (vitamin D)	+	0.30 „	6.00	255
	„ 3 ***) (xanthophylls)	+	0.30 „	3.95	113
B	Calciferol		0.15 I.U.	1.60	
	„		0.30 „	3.35	
	„		0.60 „	5.15	
	Fraction 1 ***) (carotene)	+	0.30 „	1.80	54
	„ 2 **) (vitamin D)			5.15	
	„ 3 ***) (xanthophylls)	+	0.30 „	2.75	78

*) amount corresponding to 5 g artificially dried grass.
**) „ „ „ 1 „ „ „ „
***) „ „ „ 3.5 „ „ „ „

It is clear from the percentage recovery figures that fraction 1 (carotene) contains the rachitogenic factor, some 50% of the added vitamin being inactivated. As anticipated, fraction 2 contained the vitamin D, while fraction 3 (xanthophylls) was inactive, having neither antirachitic nor rachitogenic activity as indicated by complete recovery of the added calciferol.

Using this method it is possible to separate, by chromatography, vitamin D from the rachitogenic factor, and thus determine the true vitamin D content of roughages. A number of samples of hay and artificially dried grass have been assayed in this manner; at the same time an assay was done by the normal technique on the non-saponifiable residue of the fat. Samples containing detectable amounts of the rachitogenic factor should have a higher potency after chromatography of the fat. Some results are shown in table 2.

Table 2. Vitamin D content of some roughages.

Sample	Without chromatography	After chromatography
Hay		
1	1375 I.U./kg	1420 I.U./kg
2	1090	1025
3	1090	1070
4	1005	955
Artificially dried grass		
1	270	595
	375	630
	345	450
	—	600
2	555	760
3	1150	1790

The vitamin D content of the hay samples was not affected by chromatography and it is concluded that these samples did not contain measurable amounts of the rachitogenic factor. This conclusion is valid only for these particular samples as we have repeatedly demonstrated in the past that hay contains the factor (WEITS, 1952). Samples 2, 3 en 4 were from the same field and differed only in the method of curing. Sample 2 was cured in windrows and cocks and samples 3 and 4 on tripods. In this experiment the method of curing had no effect on the vitamin D content of the hay.

The situation is quite different for artificially dried grass where higher vitamin D potencies were found after chromatography. The samples, therefore, contain considerable amounts of the rachitogenic factor. This has always been our experience and it may be concluded that the factor is always present in artificially dried grass. Carotene was not determined in the samples assayed for vitamin D. Since we have found that the rachitogenic factor is associated with carotene in roughages, marked variations in their carotene content would be in keeping with the variations noted in the rachitogenic activity of different samples of hay assayed at various times. BARTLETT et al. (1938) found that the carotene content of hay varied with the method of curing and was considerably less than in artificially dried grass; all samples being made from the same pasture.

Since hay does not always contain the rachitogenic factor while artificially dried grass does, sometimes in large amounts, it might be concluded that the factor is destroyed to a greater or lesser extent during curing. Another, but less likely, explanation is that, since on the average hay contains more vitamin D than artificially dried grass, the effect of the rachitogenic factor is masked in hay where the ratio between the factor and vitamin D is changed in favour of the vitamin.

From a practical point of view in livestock feeding it is an important question to know the ratio between the antirachitic and rachitogenic properties of a crop, provided that one can consider the antagonism on such a basis. The question is all the more important since the "antagonism" in hay and artificially dried grass will probably also be present in pasture. This is supported by findings in New Zealand (EWER, 1948). The question cannot be answered yet but it is clear that the vitamin D activity of forages is inactivated to an appreciable extent by the rachitogenic factor. It is not certain whether the rachitogenic effect is the same for the rat as for cattle. Since a rat assay gave a vitamin D potency of 800–1200 I.U./kg for green oats which caused rickets in sheep it might be concluded that sheep are more sensitive to the rachitogenic factor than the rat; the same tendency to higher sensitivity has been observed in guinea pigs (EWER, 1950). The rachitogenic factor in pasture may be of significance in the frequent occurrence of rickets in foals on pasture. It may also be one of the determining factors in the occurrence of grass tetany in cows.

The possibility that the rachitogenic factor is a phytosterol capable of combining with digitonin has been considered. Since it is known that phytosterols are very poorly absorbed this possibility is not great. Moreover, one would not expect sterols to be eluted with carotene. The absorption spectrum (fig. 1) clearly demonstrates that fraction 1 contains the carotene (DEYS, 1951).

To check this point such phytosterols were removed from the non-saponifiable fraction of the grass fat with digitonin. The regression line of the fat

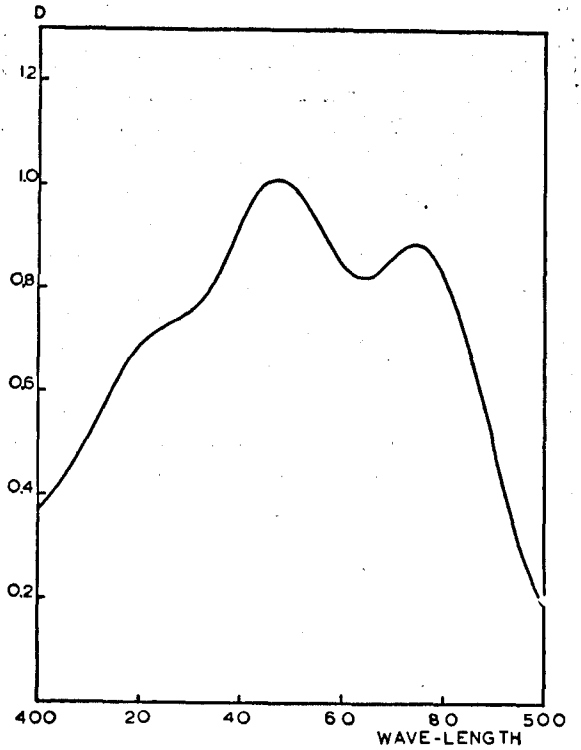

FIG. 1. ADSORPTION SPECTRUM OF FRACTION 1 IN PETROLEUM ETHER B.P. 40–60 °C.

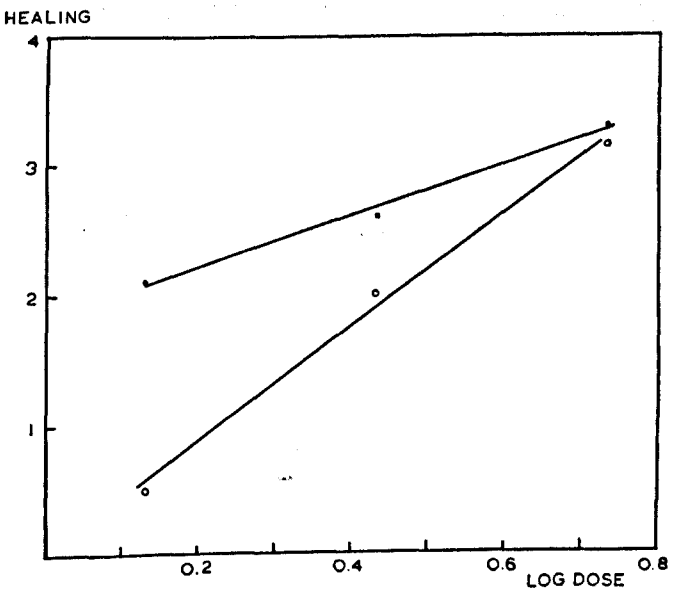

FIG. 2. VITAMIN D STANDARD (o–o–o) AND GRASSFAT (.–.–.) REGRESSION LINES.

Lower grassfat dose : 675 I.U. vitamin D/kg.
Middle „ „ : 440 „ „ „/„
Higher „ „ : 315 „ „ „/„

treated in this way has a smaller slope than the regression line of pure vitamin D (fig. 2), the difference being highly significant. Hence, it may be concluded that the rachitogenic factor is not a digitonisable phytosterol.

Meantime it has been announced recently that the rachitogenic factor is carotene itself (GRANT, 1953). This is very much in keeping with our results.

It has been known for a long time that a physiological antagonism exists between vitamins A and D (VEDDER, 1938), although the observation that this antagonism may lead to the occurrence of rickets is new. RODAHL (1950) reports a narrow epiphyseal line in rats after the administration of large doses of vitamin A. A well known consequence of a toxic dose of vitamin A is the occurrence of spontaneous fractures caused by a bone deformity resembling that of the disease described by VON RECKLINGHAUSEN (COLLAZO, 1933).

The significance of the antagonism, found in roughages not only lies in the occurrence of detectable abnormalities when large amounts of carotene are present (GRANT, 1953) but also in the fact that moderate amounts of carotene depress the antirachitic activity of roughages. How far this may elucidate some problems in animal nutrition has yet to be determined.

LITERATURE

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