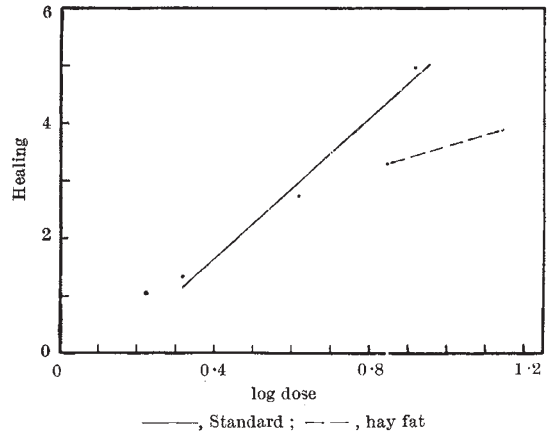
A Factor in Hay inhibiting the Action of Vitamin D

DURING our investigation on the vitamin D content of hay in Holland, started in 1950, the impression soon arose that hay contains a factor antagonistic to vitamin D.

It was noticed repeatedly that the regression line, indicating the relation between recovery of rachitic rats and the logarithm of the dose of pure vitamin D, showed a higher—sometimes a substantially higher—regression coefficient than the analogous regression line of the fat from hay under examination (see graph). Doubling the dose of hay fat scarcely ever resulted in the recovery which would be anticipated from the initial dose applied.



Statistical calculation showed that the difference in slope of the lines for each individual experiment was not at all significant; in other words, the regression lines could be considered as running parallel.

As more experimental results became available, we were able to find out whether the two groups of regression coefficients indicated real differences. The results are summarized in Table 1. Only those experimental results have been inserted for which it could be assumed that they were on the straight part of the sigmoid curve indicating the relation between the log-dose and recovery.

Table 1

Exp. No.	Regression coefficient		Difference
	Standard	Hay fat	
1	3·87	1·46	− 2·41
6	6·12	1·99	− 4·13
7	5·31	2·99	− 2·32
	5·31	2·99	− 2·32
8	6·10	4·32	− 1·78
11	4·07	3·16	− 0·91
13	6·56	3·46	− 3·10
	6·56	7·65	+ 1·09
14	5·27	2·09	− 3·18

Mean difference, $\bar{x} = 2{\cdot}12$; standard error, $s_{\bar{x}} = 0{\cdot}50$; t(exp.) = 2·12/0·50 = 4·240 ; t(theor.) ($P = 0{\cdot}01$) = 3·355.

It was evident from this that there was a highly significant difference between the regression coefficients of the standard and the hay-fat regression lines.

To prove in another way also that hay contains an anti-vitamin D substance, we have, from May to October 1951, added to hay fat definite quantities of vitamin D and investigated whether these could be recovered. We have noticed in the meantime that we have followed thereby the same course as Grant[1], and we arrived at exactly the same results.

During a fortnight, a few groups of eleven rats each were fed daily a quantity of hay fat, corresponding to 0·6 gm. of hay, contained in 0·2 c.c. of arachis oil. Other groups were given additionally 0·3 I.U. of vitamin D in the hay-fat solution. We were never able to recover the extra quantity of vitamin D supplied in that way. The quantity lost varied from 20 to 50 per cent. Neither was it recovered if the vitamin D and the hay fat were given separately at an interval of 8 hr., by supplying the vitamin D in the morning and the hay fat at night, and allowing the rats to take food during the day (Table 2). The inhibiting action of hay, therefore, cannot be attributed to a decreased absorption or destruction of vitamin D in the digestive tract, but is effective in the body after resorption from the gut.

Table 2

Exp. No.	Daily dose	X-ray healing	Vitamin D based on healing effect	Deficit (per cent)
10*A*	Fat of 0·6 gm. hay in 0·2 c.c. oil	1·56	1·98 I.U.	
	Fat of 0·6 gm. hay with 0·3 I.U. vit. D in 0·2 c.c.	3·32	5·28 ,,	21
10*B*	Fat of 0·6 gm. hay in 0·2 c.c. oil	2·80	3·96 ,,	
	Fat of 0·6 gm. hay with 0·3 I.U. vit. D in 0·2 c.c.	3·82	7·04 ,,	27
16	Hay fat in 0·2 c.c. oil	2·63	4·56 ,,	
	Hay fat with 0·4 I.U. vit. D given together	3·96	8·21 ,,	35
	Hay fat and 0·4 I.U. vit. D given separately	3·67	7·21 ,,	53

The antivitamin D factor (which we assume to be the same as that found by Grant) must be rather stable, as it has been found in hay as well as in dried grass. Furthermore, it is not only soluble in ether but also in petroleum-ether (b.p. 40–60° C.). The factor is resistant to saponification, as we could demonstrate it in the unsaponifiable fraction of hay fat.

The discovery of this factor inhibiting vitamin D may offer a partial explanation of the fact that some authors have not been able to find any vitamin D in fresh and artificially dried grass, whereas others could, and also of the unexplainable large differences in the vitamin D content sometimes noticeable in hay.

It is too early yet to offer an opinion on the importance of the anti-vitamin D factor as regards practical animal feeding. Our experiments with rats show in any event that the action of vitamin D can be diminished by 20–50 per cent by the factor. It is not at all certain that with cattle, for example, the effect will be exactly the same. That the existence of the factor can raise difficulties in practical feeding is clear from the observations on sheep recorded by Fitch[2] and Ewer[3] in New Zealand.

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[1] Grant, A. B., *Nature*, **168**, 789 (1951).
[2] Fitch, L. W. N., *Aust. Vet. J.*, **19**, 2 (1943).
[3] Ewer, T. K., *Brit. J. Nutr.*, **2**, 406 (1948); *Nature*, **166**, 732 (1950).